General Biochemistry

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بسم الله الرحمن الرحيم

﴿وفوق كل ذي علم عليم﴾



إهـــــداء

إلى من علمنى الحياة ....

إلى من ملأ قلبى حبا للمعروف والإحسان

ليكون مصباحا أستضئ به فى حياتى ....

إلى من احتواه ثرى مكــة المكـــرمـــة............

إلى والدى الحاج/ عبدالمقصود على عبد المقصود

عليـــه رحمـــة الـلـــه وبركــاتـــــه..

[وقل ربِّ ارحمهما كما ربيانى صغيرا]

PREFACE TO THE FIRST EDITION

In the name of Allah, the benificial, the merciful and most gracious.

In the first edition of review of general biochemistry book 1, I have done all I can to simplify it in the best possible way. All the formulae were redone. All efforts have been done to upgrade the text avoiding as much as possible most of the complications.

As this book is written mainly for professionals, whether students or doctors, it was through that paying attention for relevant medical proplems might be of importance.

I hope that my readers will be happy and can easily understand the knowledge offered for them in this book.

Dr. Hussein Abdel-Maksoud AL

د/ حسين عبد المقصود

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ACIDS AND BASES



ACIDS AND BASES

Acid:

It is hydrogen ion donor (it has "H" at its first element) *e.g.* HCl, H_2SO_4, H_2CO_3.

Base:

It is hydrogen ion acceptor (it has metal ion at its first element) *e.g.* NaOH.

Salt:

It is a chemical formed by the positive ion of a base and the negative ion of an acid.

$$H^+Cl^- + Na^+OH^- \longrightarrow Na^+Cl^- + H_2O$$

Also, the negative ion of the base (OH^-) combines with the positive ion of the acid (H^+) to produce water.

When an equal amount of an acid and a base react (H^+ = OH), there will be no acid or base and there will be only salt and H^2O. So, the solution is netural (acid neutralizes the base) *i.e.* nutralization reaction.

Concentration and strength of acids and bases:

Concentration:

It refers to the amount of solute per liter of solution *e.g.* 0.1 N solution of HCl or H_2SO_4 or HNO_3 is of the same concentration.

Strength:

The strength of an acid is its ability to ionize; its readiness to donate protons to water.

So, strong acids are highly ionized *e.g.* HCl and H_2SO_4, while weak acids are slightly ionized in solution *e.g.* acetic acid which is present always as CH_3COOH and a small amount is present as $CH_3COO^- + H^+$.

The same is applied to bases:

Strong bases are always ionized *e.g.* NaOH and KOH, while weak bases are slightly ionized as NH_4OH which is present always in non-ionized form and a small amount is present as $NH_4^+ + OH^-$.

A strong acid or a strong base is always strong regardless the concentration of the solution.

Hydrolysis of salts:

Solution of salts are of different reactions. Many salts are not neutral in water.

* *NaCl is neutral, it is produced by following reaction:*

Na^+OH^- + H^+Cl^- ⟶ Na^+Cl^- + H_2O

Strong base   **Strong acid**   **It is ionized but + ve ions = - ve ions**

* *Na_2CO_3 is basic, it is produced by the following reaction:*

NaOH + H_2CO_3 ⟶ $NaCO_3$ + $2H_2O$

Strong base   **Weak acid**

* In solution, Na_2CO_3 gives rise to H_2CO_3 which is not ionized (weak acid) and Na^+OH^- which is ionized (strong base).
* *NH_4Cl (ammonium chloride) is acidic in solution, it is produced by the following reaction:*

NH_4OH + H^+Cl^- ⟶ NH_4Cl + H_2O

Weak base   **Strong acid**

N.B.

Hydrolysis is a reaction in which H_2O participates in breaking down of a substrate (= breakdown by H_2O).

INDICATORS

These are chemical (dyes) which change their colours at different pH. They give one colour in acidic medium and another colour in alkaline medium.

Indicator	Acid	Base	pH Range
Litmus paper	Red	Blue	-
Phenolphthalin	Colourless	Magenta pink	3.8-10.0
Topfer's reagent	Red	Yellow	2.9-4.0

Determination of the concentration of a solution of a base or acid by using indicators:

Determination of concentration of NaOH:

1. Take 5 ml NaOH solution in a beaker and add to it 2 drops phenolphthalein. This will give magenta red colour.
2. Take 0.1 N HCl solution and put it in a burette.
3. Add HCl to NaOH drop by drop while shaking.
4. The first drop that turns the solution in the beaker colourless is the end point.
5. Calculate the amount of HCl taken to neutralize the 5 ml of NaOH (*e.g.* 7.5 ml).
6. Use the following equation to determine the concentration of NaOH.

$$C_1 \times V_1 = C_2 \times V_2$$

Where:

C_1 = Concentration of HCl = 0.1 N.

V_1 = Volume of HCl taken to neutralize 5 ml NaOH.

C_2 = Unknown concentration of NaOH.

V_2 = 5 ml NaOH.

$$C_1 \times V_1 = C_2 \times V_2$$

$$0.1 \times 7.5 = ? \times 5$$

So, concentration of NaOH = [(0.1x7.5)/5] = 0.15 N

Determination of acidity in gastric juice:

1. Take a known volume of gastric juice in a beaker *e.g.* 5 ml.
2. Add 2 drops of Topfer's reagent (red in acid).
3. Put 0.1 N NaOH in a burette and take the reading of the scale.
4. Add the NaOH drop by drop to the gastric juice while shaking until the first drop that turns the solution yellow.

5. Take the reading of the burette and calculate the amount of 0.1 N NaOH taken by 5 ml of gastric juice (e.g. 3 ml).

$$C_1 \times V_1 = C_2 \times V_2$$

$$0.1 \times 3 = ? \times 5$$

So, concentration of HCl in gastric juice = [(0.1x3)/5]
= 0.06 N

pH = REACTION

Definition:

pH is the potency of hydrogen ion (p = potency, H = hydrogen), or it is the measure of hydrogen ion concentration. Also, it may be defined as negative log of hydrogen ion concentration (H^+).

The scale of pH is from 1-14:

When pH = 7, it is netral as the number of (H^+) = (OH^-),

When pH is less than 7 (1-7), it is acidic (H^+ OH^-),

When pH is more than 7 (7.1-14), it is basic (OH^- H^+).

A solution of pH 1 contains $1x10^{-1}$ moles H^+/L and 10^{-13} moles OH^-/L (Acidic).

A solution of pH 7 contains $1x10^{-7}$ moles H^+/L and 10^{-7} moles OH^-/L (Neutral).

A solution of pH 1 contains $1x10^{-12}$ moles H^+/L and 10^{-2} moles OH^-/L (Basic or alkaline).

0.1 N HCl contains $1x10^{-1}$ moles H^+/L (pH = 1), while 0.01 N HCl contains $1x10^{-2}$ moles H^+/L (pH = 2).

Distilled water is of neutral pH (pH = 7). When it is left in the open air, it takes CO_2 producing some H_2CO_3 (carbonic acid) and becomes slightly acidic.

$$H_2O + CO_2 \longrightarrow H_2CO_3$$

Calculation of pH:

- 0.1 N solution contains $1x10^{-1}$ (H^+), so its pH = 1.
- 0.2 N solution is of pH = $2x10^{-1}$. its pH is calculated as follows:
  Log of 10^{-1} = 1, log of 2 = 0.3,
  So, pH of 0.2 N solution = 1-0.3 = 0.7.
- 0.0003 N solution is of (H^+) = $3x10^{-4}$
  Log of 10^{-4} = 4, log of 3 = 0.48
  pH of 0.0003 N solution = 4 - 0.48 = 3.52
- 0.002 N solution is of (H^+) = $2x10^{-3}$
  Log of 10^{-3} = 3, log of 2 = 0.3
  pH of 0.002 N solution = 3 – 0.3 = 2.7

SOLUTION

The concentration of the solution can be expressed as the following:

% (Percentage):

* % weight/volume w/v (the most commonly used).
* % v/v for liduids.
* % W/W for solids.

e.g. 1% NaCl = 1 g in 100 ml solvent.

Molar solution "M":

- It is the molecular weight in grams dissolved in one liter solvent.
- Molar solution of NaOH =
  Molecular wt of Na + O + H = 23 + 16 + 1 = 40
  molar solution of NaOH contains 40 g/L.
- 2 molar solution of NaCl:
  Molecular wt of Na + Cl = 23 + 35 = 58
  So, 2 molar solution of NaCl = (58 x 2) g/L = 116 g/L.

Normal solution "N":

- The equivalent weight in grams dissolved in one liter solvent (*i.e.* normality = one g Eq/L).
- The equivalent wt = [Molecular wt / Valency].
- If valency is 1, Molarity = Normality *e.g.* a normal solution of HCl is also a molar solution, while a normal solution of H_2SO_4 is a 0.5 molar solution.

Molal solution:

A molal solution contains 1 mole of the solute in 1000 g of solvent.

Ions:

These are atoms that gain or loose electrons:

- Anions (-ve) atom that gained electron.
- Cation (+ve) atom that lost electron.

Examples of chemical bonds:

1. Ionic bond:

One atom gives electrons to another. The atom that lost electrons becomes +ve (cation) and that gained electrons becomes -ve (anion). Then both of them are attracted by the different charges → ionic bond.

Compounds having ionic ionize in solution → electrolyte solution (electrically charged). They give +ve ions that combine with -ve ions of the solvent (e.g. OH^- in H_2O) and give also –ve ions that combine with +ve ions of the solvents (*e.g.* H^+ of H_2O).

2. Covalent bonds:

No gain of loss of electrons but both atoms share electrons in their outer level. Compounds having covalent bonds dissolve but do not ionize in solution.

PUFFERS

Definition:

These are substances that present pH changes in a solution.

Composition:

- A buffer is composed of a mixture of a weak acid and its salt with a strong base *e.g.* H_2CO_3 and $NaHCO_3$.
- Or it is a mixture of a weak base and its salt with a strong acid *e.g.* NH_4OH and NH_4Cl.

Mechanism of action:

H_2CO_3 for an example, is weak acid. It donates only one hydrogen ion at a time (H^+, HCO_3^-). When it donates the first H^+, it becomes HCO_3^- (bicarbonate). When H_2CO_3 reacts with NaOH (strong base) it gives $NaHCO_3$.

H_2CO_3 + $NaHCO_3$ constitute a buffer pair that keeps pH of the body.

Physiological buffer systems:

Enzymes, which control metabolic processes, are very sensitive to changes in pH. So any acid or base formed inside the body should be rapidly and effectively buffered to allow such processes to proceed.

The pH of the human body ranges from 7.35-7.45. This means that the body fluid (*e g* blood) is slightly alkaline.

I. Bicarbonate system:

H_2CO_3 / $NaHCO_3$ in 1 : 20. It is the most important buffer system in the body because:

a) It is easily produced from CO_2 of metabolic reaction.
b) It is easily excreted by the action of carbonic anhydrase in the lungs as CO_2.

II. Protein system:

Proteinic acid/proteinate salt.

III- Phosphate system:

Acid phosphate/alkaline phosphate, as K dihydrogen phosphate/Na monohydrogen phosphate.

There are 3 salts formed when H_3PO_4 reacts with NaOH or KOH:

1. NaH_2PO_4 Sodium dihydrogen phosphate most acidic.
2. Na_2HPO_4: Sodium monohydrogen phosphate.
3. Na_3PO_4: Sodium phosphate (least acidic).

Blood buffers:

Blood buffers include all the physiological buffers mentioned above. In the plasma the base is Na^+, while in red blood cells it is mainly K^+. The protein system present include the plasma proteins (albumin, globulins and fibrinogen) and haemoglobin (H.Hb/K.Hb) and oxyhaemoglobin $H.HbO_2$/ $K.HbO_2$) in red cells.

Haemoglobin, oxyhaemoglobin systems are responsible for the buffering of most of CO_2 added to the blood by tissues.

ACIDOSIS

Definition:

It is a condition in which pH of blood tends to decrease due to increase ratio of $H_2CO_3/NaHCO_3$.

It usually results from the formation or absorption of acids at a rate exceeding that of their neutralization and elimination. Also, it may result from loss of bases from the body.

Concentration:

Acidosis can be classified from the clinical point of view into 2 types:

1. Respiratory acidosis:

It is not of immediately serious concern because CO_2 diffusion through the lung tissues into alveolar air is 25 times as rapidly as CO_2.

Respiratory acidosis is caused by an increase in H_2CO_3 relative to $NaHCO_3$. This may occur in any disease that impairs respiration such as:

1. Depression of respiratory center by drugs as morphia.
2. Obstruction of air passages.
3. Pulmonary disease.
4. Excessive breathing of CO_2.

If the pH of the blood is not changed inspite of acidosis, this is called compensated acidosis. In acidosis there is rapid respiratory rate to wash out excess CO_2 from the lung. CO_2 stimulates the respiratory center in the brain.

When the alkali reserve is exhausted i.e., when all HCO_3^- is taken by H^+, there will be change of pH of the vlood → uncompensated acidosis which must be corrected immediately.

2. Metabolic acidosis:

It is caused by a decrease in HCO_3 fraction with either no change or a relatively smaller change in H_2CO_3 fraction. This is the most classical type of acidosis.

Causes:

a) increased production of acids: e.g.:

1. Excessive intake of protein → increased production of phosphoric, sulfuric and uric acids.
2. Severe muscular exercise → increased lactic acid.
3. Ketosis as in low CHO diet, prolonged starvation, and uncpntrolled diabetes mellitus.

b) Failure of exerction of acids: e.g. renal failure.

c) Increased loss of bases: e.g.

1. Severe diarrhea.
2. Vomiting due to low intestinal obstruction.
3. Hyperkalaemia.

Acidaemia: It is a condition in which pH of the blood decrease.

Treatment of acidosis:

The chief measures are:

1) Treatment of the disease causing acidosis.
2) Replacement of lost base and H_2O by:
   a) Administration of alkaline buffers such as Na lactate, and $NaHCO_3$.
   b) Supplying fruit juices, milk, and vegetables in diet.

ALKALOSIS

Definition:

It is a condition in which pH of the blood tends to increase due to decrease ratio of $H_2CO_3/NaHCO_3$. Primary alkali excess or increase in alkali reserve is the most frequent cause of clinically observed alkalosis.

Classification:

1. Respiratory alkalosis:

Occurs when there is a decrease in H_2CO_3 fraction with no corresponding change in $NaHCO_3$. Hyperventilation causes loss of CO_2 and decrease in H_2CO_3 in blood *e.g.* fever, high attitude and hysterical attacks.

2. Metabolic alkalosis:

Occurs when there is increase of $NaHCO_3$ fraction with either no change or relative smaller change in carbonic acid fraction. It is due to:

a) Increased loss of acids, e.g. in:

- Repeated gastric suction.
- Vomiting due to high intestinal obstruction.
- Hypokalaemia.

b) Increased administration of bases, e.g. in:

- Large amounts of alkalies in diet.
- Administration of Na and K citrate and bicarbonate.

Alkalaemia: It is a condition in which pH of the blood increases.

Treatment of alkalosis:

- administration of HCl by mouth.
- Inhalation of CO_2.
- NH_4Cl injection.

OSMOSIS

Definition:

When 2 solutions are separated by semipermeable membrane (a membrane that allows free passage of H_2O); the solvent will pass from solution of lower concentration to the higher concentration till the hydrostatic pressure of the latter prevents further passage of the solvent.

Osmotic pressure:

It is the pressure required to prevent any net movement of solvent between a solution and its pur solvent when they are separated by a semipermeable membrane.

This osmotic pressure is due to unequal bombardment of the membrane on its opposite sides by the solvent molecules.

The O.P. is directly proportionate to the concentration of solution, number of molecules or ions in solution and temperature.

Solutions are classified into types according to their osmotic pressure:

1. Isotonic solutions have O.P. equal to that of body fluid.
2. Hypertonic solutions have higher O.P.
3. Hypotonic solutions have lower O.P.

O.P. plasma proteins is called oncotic pressure = 30 mmHg.

Physiological significance of osmotic pressure:

1. Formation and reabsorption of interstitial fluid:

The interstitial fluid is formed by filtration of the blood plasma at the arterial end of the blood capillaries and is reabsorbed at the venous end. This process is chiefly governed by the capillary blood pressure and the O.P. of the plasma proteins.

2. Volumes of body fluids:

The body regulates the volume of the various body fluids according to their content of solutes so as to maintain their osmotic pressure constant.

3. Formation of urine:

The formation of urine starts by filtration of blood plasma through the glomerular capillaries into the Bowman's capsule. This process is chiefly governed by the capillary blood pressure and the O.P. of the plasma proteins.

4. Haemolysis:

The red blood cells are isotonic with 0.9 g% NaCl solution, but if put in hypertonic solution, they loss H_2O and become crenated. On the other hand, if put in hypotonic solution they absorbe H_2O and swell → rupture.

CARBOHYDRATES

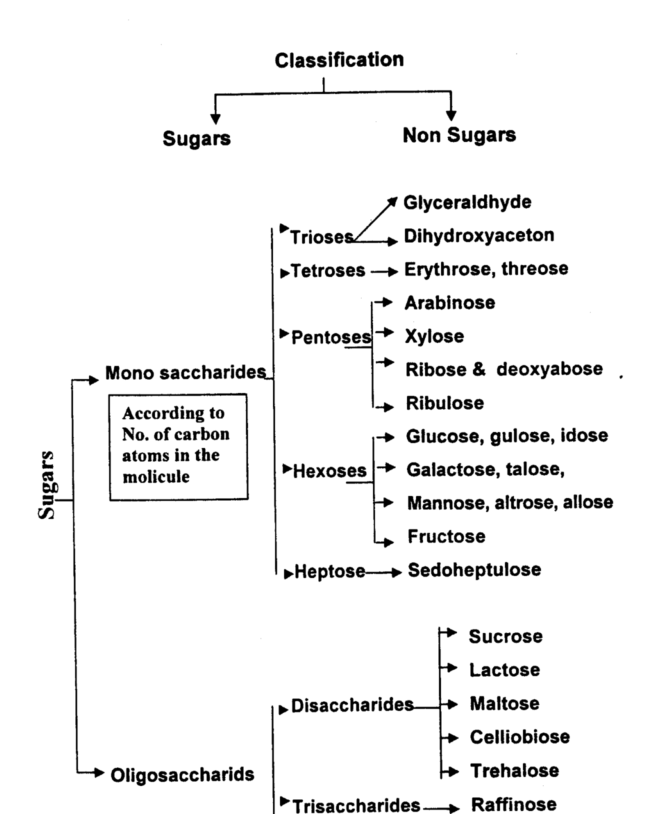
CARBOHYDRATES

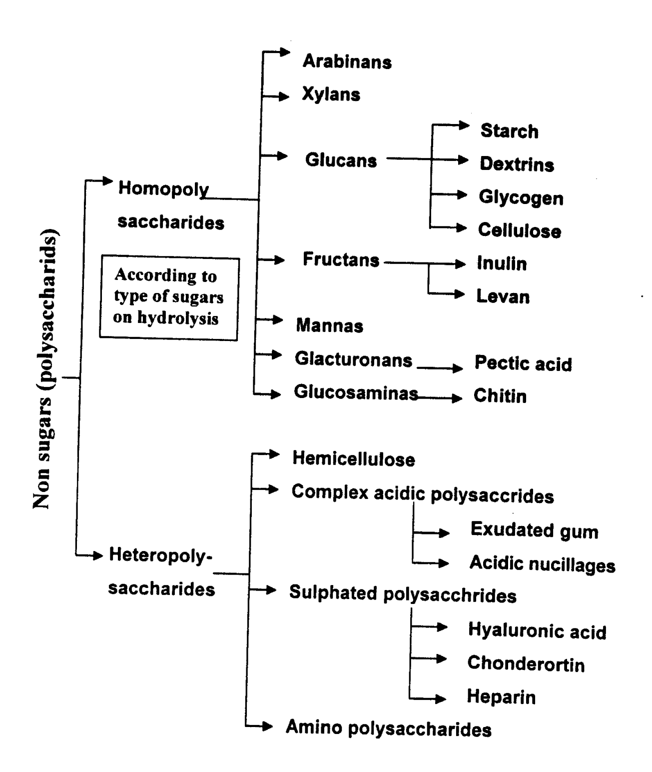
Definition:

- Organic chemical mainly neutral compounds make up most of the structure of plant as in some extent animals tissues containing carbon hydrogen and oxygen in a ratio that of water.
- Have the empirical formula C_X $(H_2O)_X$ e.g. $C_6H_{12}O_6$ which is generally but not always some non carbohydrates have the some formula e.g. acetate $C_2H_4O_2$, lactic acid $C_3H_6O_3$ and some carbohydrates not follow that formula e.g. deoxyribose $C_5H_{10}O_4$, rhamnose $C_6H_{12}O_5$.
- May defined also as polyhydroxy aldhydes or ketons.
- Their reduction products are polyhydricalcohols.
- Their oxidation products are aldonic, uronic and aldaric acid.
- Their substitution products as amino, sulpho sugar.

Biological importance:

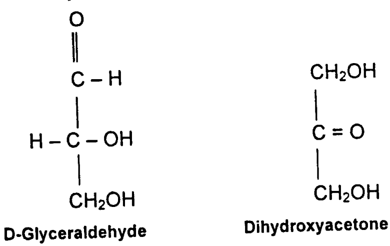
- The chief function in the animal organisms is that a fuel in all mammals except ruminants its degradation parts of which CO_2, H_2O represent a source of energy.
- Certain products of its metabolism act in breakdown of many food stuffs as catalysts or promote oxidation.
- Act as starting materials of biological synthesis of other types of compounds as fatty acids, amino acids and steroids.
- Inter in the structure of certain biological important compound as glycoproteins, nuclic acid.
- There are diseases related to carbohydrates as diabetes mellitus, galactosaemia and glycogen storage diseases


Classification
Sugars
Non Sugars
Sugars
Mono saccharides
According to No. of carbon atoms in the molicule
Trioses
Glyceraldhyde
Dihydroxyaceton
Tetroses
Erythrose, threose
Pentoses
Arabinose
Xylose
Ribose & deoxyabose
Ribulose
Hexoses
Glucose, gulose, idose
Galactose, talose,
Mannose, altrose, allose
Fructose
Heptose
Sedoheptulose
Oligosaccharids
Disaccharides
Sucrose
Lactose
Maltose
Celliobiose
Trehalose
Trisaccharides
Raffinose
Tetrasaccharids
Stachyose


Non sugars (polysaccharids)
Homopoly saccharides
According to type of sugars on hydrolysis
Arabinans
Xylans
Glucans
Starch
Dextrins
Glycogen
Cellulose
Fructans
Inulin
Levan
Mannas
Glacturonans
Pectic acid
Glucosaminas
Chitin
Heteropoly-saccharides
Hemicellulose
Complex acidic polysaccrides
Exudated gum
Acidic nucillages
Sulphated polysacchrides
Hyaluronic acid
Chonderortin
Heparin
Amino polysaccharides

Monosaccharides:

- Monosaccharides have the empirical formula $(CH_2O)_n$, where n $\leq$ 3 or some larger number.
- The carbon skeleton of the common monosaccharides is unbranched and each carbon atom except one, contains a hydroxyl group.
- At the remaining carbon there is a carbonyl oxygen, which is often combined in an acetal or ketal linkage.
- If the carbonyl group is at the end of the chain, the monosaccharide is an aldehyde derivative and called an aldose, if it is at any other position, the monosaccharide is a ketone derivative and called a ketose.
- The simplest monosacchrides are the three carbon trioses, glyceraldehyde and dihydroxyacetone.



D-Glyceraldehyde **Dihydroxyacetone**

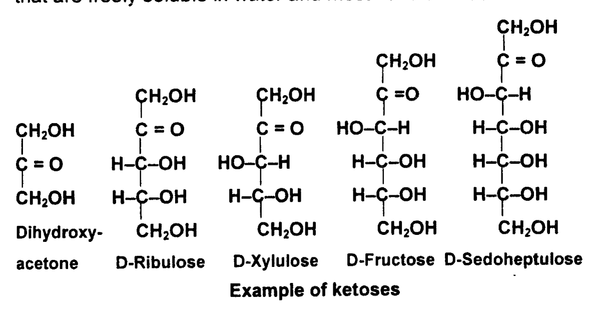
Glyceraldehyde is an aldotrios, while dihydroxy-acetone is a ketotriose.

Also among the monosaccharides are the tetroses (four carbons), pentoses (five carbons), hexoses (six carbons), heptoses (seven carbons) and octoses (eight carbons). Each exists in two series, i.e. aldotetroses and ketotetroses, aldopentoses and ketopentoses, aldohexoses and ketohexoses ... etc.

In both classes of monosaccharides the hexoses are by far the most abundant.

However, aldopentoses are important components of nucleic acids and various polysaccharides. Also, derivatives of trioses and heptoses are important intermediates in carbohydrate metabolism.

All the simple monosaccharides are white crystalline solids that are freely soluble in water and most have a sweet taste.



Example of ketoses

D-Glyceraldehyde	D-Erythrose	D-Ribose	D-Xylose
CHO	CHO	CHO	CHO
H–C–OH	H–C–OH	H–C–OH	H–C–OH
CH₂OH	H–C–OH	H–C–OH	HO–C–H
	CH₂OH	H–C–OH	H–C–OH
		CH₂OH	CH₂OH

D-Glucose	D-Mannose	D-Galactose	D-Altrose
CHO	CHO	CHO	CHO
H–C–OH	HO–C–H	H–C–OH	HO–C–H
HO–C–H	HO–C–H	HO–C–H	H–C–OH
H–C–OH	H–C–OH	HO–C–H	H–C–OH
H–C–OH	H–C–OH	H–C–OH	H–C–OH
CH_2OH	CH_2OH	CH_2OH	CH_2OH

D-Talose	D-Idose	D-Glucose	D-Allose
CHO	CHO	CHO	CHO
HO–C–H	HO–C–H	H–C–OH	H–C–OH
HO–C–H	H–C–OH	H–C–OH	H–C–OH
HO–C–H	HO–C–H	HO–C–H	H–C–OH
H–C–OH	H–C–OH	H–C–OH	H–C–OH
CH_2OH	CH_2OH	CH_2OH	CH_2OH

Examples of aldoses

Stereoisomerism of monosaccharides:

All the monosaccharides, except dihydroxyacetone, contain one or more asymmetric carbon atoms (As asymmetric carbon atom is the carbon atom to which 4 different atoms or groups of atoms are attached).

The presence of asymmetric carbon atoms in a compound makes possible the formation of isomers of that compound.

Glyceraldhyde contains only one asymmetric carbon atom and therefore exists as two different stereoisomers. D- and L-glyceraldehyde are considered to be the reference, or parent,

compounds for designating the configuration of all stereoisomeric compounds.

```
    O                   O
    ||                  ||
    C-H                 C-H
    |                   |
  H-C-OH             HO-C-H
    |                   |
    CH2OH               CH2OH
```

D-Glycerose (D-glyceraldehyde) **L-Glycerose (L-glyceraldehyde)**

D and L forms of momosaccharides:

The orientation of the H and OH groups around the carbon atom just adjacent to the terminal primary alcohol carbon (e.g. carbon atom No. 5 in glucose) determines the family to which the sugar belongs. When the OH group on this carbon is on the right, the sugar is a member of the D-series; when it is on the left, it is a member of the L-series. The majority of the monosaccharides occurring in the body are of the D-configuration.

The cellular enzymes acted on D- form only.

Sterioisomerism:

These compounds are similar to each other in that they contain the same chemical groups or atoms but differ in the arrangement of these groups.

Aldotetroses have two asymmetric carbon atoms and aldopentoses have three, while aldohexoses have four.

The number of different stereoisomers of an aldose can be represented by the formula 2^n, where n = number of the asymmetric carbon atoms of that aldose.

Thus, an aldohexoses having 4 asymmetric carbon atoms can exist in the form of 16 (2^4) different steroisomers, 8 of which belong to the D-series, and the mirror images of each of these would comprise the L-series.

D-Glucose	L- Glucose
CHO	CHO
H–C–OH	HO–C–H
HO–C–H	H–C–OH
H–C–OH	HO–C–H
H–C–OH	HO–C–H
CH_2OH	CH_2OH

Optical isomerism of monosaccharides:

The presence of asymmetric carbon atoms also conffers optical activity on the compound.

When a beam of polarized light is passed through a solution exhibiting optical activity, it will be rotated either to the right or to the left according to the type of the optical isomer which is present (the polarized light is the light vibrating in one plane only, while the ordinary light vibrates in many planes).

- All monosaccharides are optically active
- A compound which causes rotation of polarized light to the right is said to be dextrorotatory and a plus (+) sign is used to designate this fact.
- Rotation of the beam of polarized light to the left (levorotating action) is designated by a minus (-) sign.
- For example, the usual form of glucose found in nature is dextrorotatory (specific rotation = + 52.7) and the usual form of fructose is levorotatory (specific rotation = -92.4).

N.B: Stereoisomerism and optical isomerism are independent properties. Thus, a compound may be designated D (-) or L (+), indicating structural relationship to D or L sugar the opposite rotation power.

Specific rotation:

$$\alpha = \frac{a \times 100}{L \times C}$$

α = angle of rotation.

L = length of tube in which the optically active substance was put in decimeter.

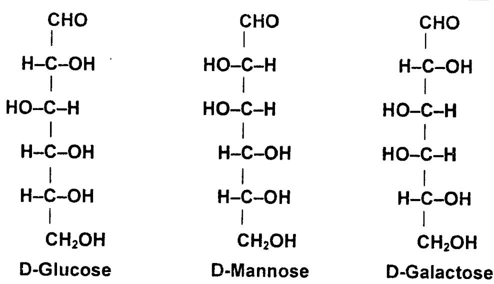
C = concentration of the optically active substance in gm/100 ml solution.

The specific rotation usually measured at 20°C.

Epimers

- Two sugars, which differ from one another only in the configuration around a single carbon atom, are termed epimers.
- Galactose and glucose are examples of an epimeric pair which differ with respect to carbon 4; mannose and glucose are epimers with respect to carbon 2.

N.B: When a mixture of two monosaccharides one is dextrorotatry and the other is levorotatory by the same angle of rotation and specific rotation this mixture is called Racemic mixture.



Mutarotation and the anomeric forms of D-glucose:

- Mutarotation is a change of optical rotation of freshly prepared sugar solution until it reaches a constant value which is called specific rotation of this sugar.

- In aqueous solutions many monosaccharides act as if they had one or more asymmetric center than is given the open-chain structural formulae,

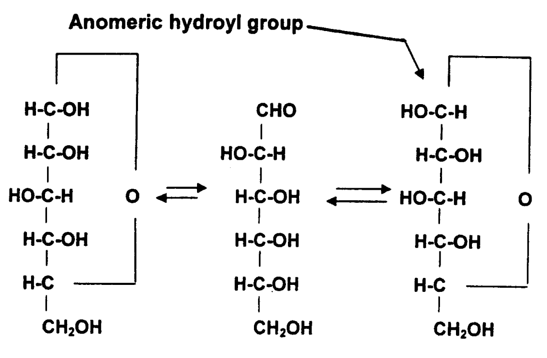
- D-glucose may exist in two different isomeric forms differing in specific rotation, α-D-glucose for which specific rotation = + 112.2 and β-D-glucose for which specific rotation = + 18.7. Both have been isolated in pure forms.

- When the α and β isomers of D-glucose are dissolved in water, the optical rotation of each gradually changes with time and approaches a final equilibrium value of specific rotation of + 52.7.

- This change, called mutarotation, which is due to the formation of an equilibrium mixture consisting of about one third α-D glucose and two thirds β-D-glucose at 20^0C.

- From various chemical considerations it had been known that the α- and β- isomers of D-glucose are not open-chain stuctures, but six-memebered ring structures called *Hawrth* formula formed by the reaction of the alcoholic hydroxyl group at carbon atom 5 with the aldehydic carbon atom 1.

- The six-membered ring forms of sugars are called pyranoses because they are derivatives of the heterocyclic compound pyran. The name for the ring form of α-D-glucose is α-D-glucopyranose.



α, D-glucopyranose D-glucose β, D-glucopyranose

N.B.:

1. If the oxygen atom united with 2 carbons in the same monosaccharided called hemiacetal linkage.
2. If the oxygen atom united with 2 different monosaccharides called acetal or glucosidic linkage.
3. If the oxygen atom united between 2 any chemical structures rather than carbohydrates called ester linkage.

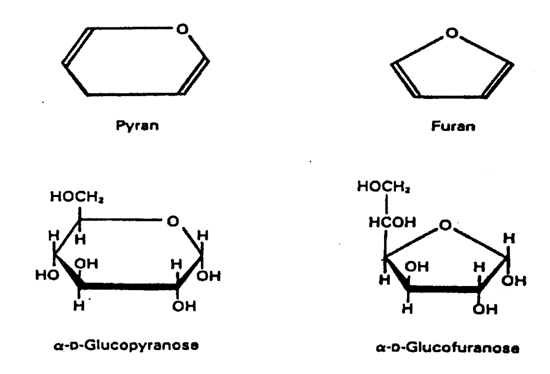
** Note also that the α form of D-glucopyranose has the anomeric hydroxyl group to the right and β fom has it to the left.

Also note that the anomeric carbon atom is distinguish from the others in being linked to two oxygen atoms.

** Isomeric forms of monosaccharides that differ from each other only in the configuration around the carbonyl carbon atom are anomers, and the carbonyl carbon atom is called the anomeric carbon.

- All aldoses with five or more carbon atoms form stable pyranose rings and can exist in anomeric forms.

Ketoses of five or more carbon atoms also occur in α and β anomeric forms. In these compounds, the alcoholic hydroxyl group on carbon atom 5 has been joined with the carbonyl group at carbon atom 2 to yield a hemiketal in the form of a five - members (Furanose)ring, a derivative of furan.



Pyranose and furanose forms of glucose.

α-D-Fructopyranose

α-D-Fructofuranose

Pyranose and furanose forms of fructose.

Important Chemical Reactions of Monosaccharides:

1. All Monosaccharides are strong reducing agents:

- The most common reagents used to test for these reducing sugars are feliling's and Benedict's solutions. Fehling A = copper sulfate solution, Fehling B = sodium hydroxide and Rochell salt (Na-K-tartarate) as catalyst.

- At the time of the test, equal volumes of Fehling A and B are mixed. This produces a dark blue, clear solution of $Cu(OH)_2$. The tartarate serves to keep the $Cu(0H)_2$ in solution. By boiling this mixture with a reducing sugar, a red brown precipitate of curprous oxide (Cu_2O) is formed.

$$CuSO_4 + 2\ NaOH \longrightarrow Cu(OH)_2 + Na_2SO_4$$

$$2\ Cu(0H)_2 + \text{Reducing sugar} \xrightarrow{Heat} 2\ CuOH + H_2O + O$$

$$2\ CuOH \longrightarrow Cu_2O + H_2O$$

Cuprous oxide (red ppt.)

- Benedict's solution contains copper sulphate, sodium carbonate and sodium citrate It is a single solution, here the alkali is weaker, so it is easy handled and more sensitive than Fehling's.

2. Oxidation of Monosaccharides to sugar acids

- There are three important types of sugar acids: aldonic, aldaric and uronic acids.

- The aldoses are oxidized at the aldehydic carbon atom by weak oxidizing agents as sodium hypoiodite or bromine water to form the corresponding carboxylic acids which are called aldonic acids, e.g. D-glucose yields D-gluconic acid. The phosphorylated form of D-gluconic acid is an important intermediate in carbohydrate metabolism.

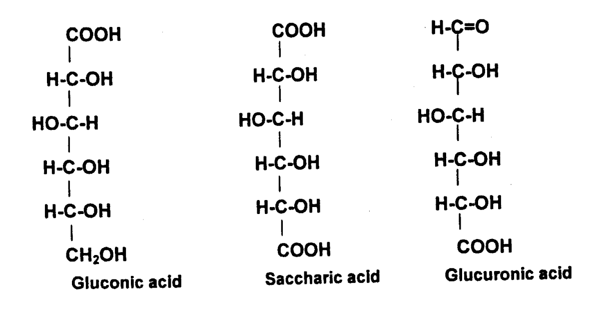
- With stronger oxidizing agent as concentrated nitric acid, both aldehydic carbon and the last carbon are oxidized to carboxyl groups yielding aldaric acids (also called saccharic acids).

- D-glucose the product is called D-glucaric acid. Aldaric acids are of no great biological significance, but sometimes useful for the identification of sugars.

- However, the third class of sugar acids, the uronic acids, *(which is an enzymatic cellular oxidation)* is biologically very important. In uronic acids, only the last carbon atom is oxidized to a carboxyl group.

- The uronic acid derived from D-glucose is D-glucuronic acid enters in the formation of certain compounds as glycoproteins. Also it is used for detoxication processes as with phenols. The bile pigment bilirubin is conjugated in the liver with glucuronic acid. Female sex hormones are excreted in urine as glucuronates.

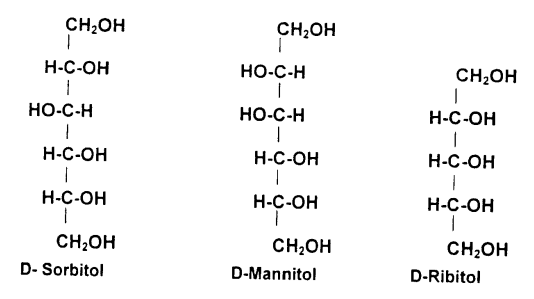
- Other important uronic acids are D-galacturonic acid and D-mannuronic acid.



Gluconic acid Saccharic acid Glucuronic acid

3. Reduction of monosaccharides to polyhydric alcohols (Sugar Alcohols)

- The carbonyl group of monosaccharides can be reduced by H_2 gas in the presence of Nickel as metal catalyst to form the corresponding sugar alcohols.
- D-glucose for example, yields the sugar alcobol D-sorbitol. D-Mannose yields D-mannitol, D-fructose gives both D-Sorbitol and D-mannitol, D-galactose gives D-dulcitol D-galacfltol).
- D-Ribose yields D-ribitol. Enzymes can also carry out such reductions.



4. Action of acids on monosaccharides:

- Strong acids act upon Monosaccharides to yield furfural derivatives, by losing three molecules of water. Pentoses give furfural and hexoses give hydroxyinethyl furfural. These compounds condense easily with alcoholic α-Naphthol one of the phenols to give coloured compounds. This reaction is used as a general test for carbohydrates. (Molisch's test)

$3H_2O$

D-Ribose Furfural

$3H_2O$

Glucose

5. Action of alkalis on monosaccharides:

- With alkalis monosaccharides react in various ways:

a) In dilute alkali : the sugar will change to the cyclic alpha and beta structures, with an equilibrium between the 2 anomers.

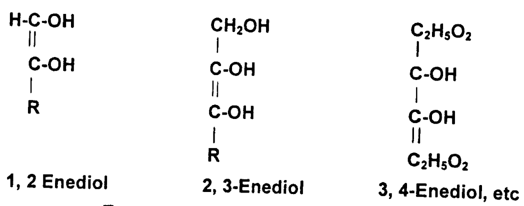
- On standing, a rearrangement will occur which produces an equilibrated mixture of glucose, fructose and mannose through the enediol form.

N.B.: Enediol means double bond between 2 carbons each of them attached with hydroxyl group

$H-C=O$
$HO-C-H$
R
Mannose

Glucose ⇌ Common enediol form of glucose, mannose, and fructose ⇌ Fructose

. If the mixture: is heated to 37°C, the acidity increases and a series of enols are formed in which double bonds shift from the oxygen-carbon link to positions between various carbon atoms.



Formation of endiols by heating

B) In concentrated alkali : sugar caramelizes and produces a series of decomposition produces.

- Yellow and brown pigments develop, salts may form, many double bonds between carbon atoms are formed and carbon-to-carbon bonds may rupture.

6. *Osazone formation:*

- Osazone formation is a useful means for preparing crystalline derivatives of the sugars. These compounds have characteristic crystal structure, melting points and are valuable in the identification of sugars.
- Osazone derivatives of sugars are obtained by adding a mixture of phenylhydrazine hydrochloride and sodium acetate to the sugar solution and heating in a boiling water bath. The reaction involves only the carbonyl carbon (i.e., aldehyde or ketone group) and the next adjacent carbon. For example, with an aldose the following reaction occurs:

HC=O + H_2N–NH–C_6H_5 (HC–OH, R) —Condensation, $-H_2O$→ HC=N–NH–C_6H_5 (HC–OH, R)

Aldose + Phenylhydrazine → Phenylhydrazone

Then the phenylhydrazone reacts with 2 additional molecules of phenylhdrazine to form the osazone.

HC=N–NH–C_6H_5 / H–C–O–H (R) + H_2N–NH–C_6H_5 —oxidation→ HC=N–NH–C_6H_5 / C=O / R + NH_3 + $C_6H_5NH_2$ (Aniline)

HC=N–NH–C_6H_5 / C=O / R + H_2N–NH–C_6H_5 —condensation, $-H_2O$→ HC=N–NH–C_6H_5 / C=N–NH–C_6H_5 / R (Osazone)

N.B. The reaction with a ketose is similar.

Sugars such as glucose, mannose and fructose which differ only in the configuration of the first two carbon atoms, give the same osazone, but the structure of galactose differs in that part of the molecule unaffected in osazone formation, it would form a different osazone.

N.B.: Glucosazone, mannosazone and fructosazone are bruch or bristles shape.

Lactosazone is sum or hedgehog shape.

Maltosazone is Rosette shape.

7. Fermentation:

- Production of ethyl alcohol enzymes present in yeast is possible.
- Fermentable sugar + yeast → CH_3-CH_2OH + CO_2

Glucose, fructose and mannose are fennented by lactose and polysaccharides are not fennentable.

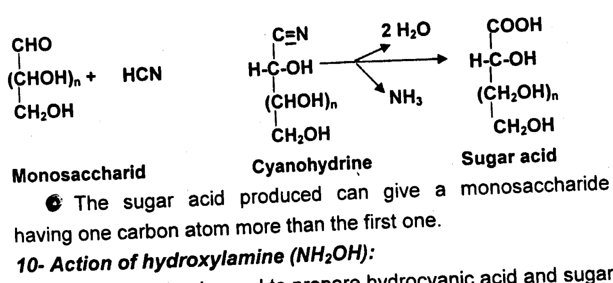
from Monosaccharides by certain yeast. Pentoses,

8. Formation of esters:

- Esters of phosphoric acids are formed in many biological reactions, as glucose-6-phosphate and glucose-i-phosphate.

9- Action of hydrocyanic acid:

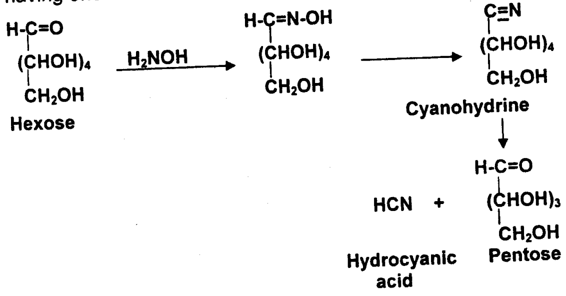
HCN is very toxic material monosaccharids react with it forming Cyanohydrine which is not toxic and hydrolyzed to the corresponding sugar acid, this reaction has a medical importance in treatment of cyanide poisoning in which an injection intravenously by hypertonic glucose solution.



● The sugar acid produced can give a monosaccharide having one carbon atom more than the first one.

10- Action of hydroxylamine (NH_2OH):

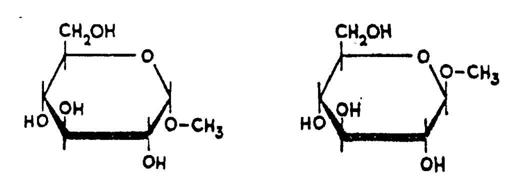
● This reaction is used to prepare hydrocyanic acid and sugar having one carbon less than the starting one.



Important Derivatives of Monosaccharides

1. Glycosides

- The glycosides are acetals formed by the reaction of the anomeric carbon atom of the intramolecular hemicetal or pyranose form of the aldohexose with a hydroxyl group furnished by an alcohol. This is called a glycosidic bond. The anomeric carbon in such glycosides is asymmetric.
- Glycosides are found in many drugs and spices and in the constituents of animal tissues.
- The aglycone (*non-carbohydrate part*) may be methanol, glycerol, phenol or a base as in adenine.
- Cardiac glycosides are important in medicine all contain steroids as the aglycone component include derivatives of digitalis and strophanthus (ouaban) an inhibitor of Na/K-ATPase.
- D-Glucose with methanol, yields methyl α-D-glucopyranoside and methyl β-D-glucopyranoside.



α-Methyl-D-glucoside β-Methyl-D-glucoside

- The glycosidic linkage is also formed by the reaction of the anomeric carbon. of a monosaccharide with a hydroxyl group of another monosaccharide to yield a disaccharide.

- Oligosaccharides and polysaccharides are chains of Monosaccharides joined by glycosidic linkages.
- The glycosidic linkage is stable to bases but is hydrolyzed by boiling with acid to yield the free monosaccharide and free alcohol.
- Glycosides are hydrolyzed by enzymes called glycosidases, which differ in their specificity according to the type of glycosidic bond (α or β), the structure of the monosaccharide unit (s) and the structure of the alcohol.

2. N-Glycosides:

- Aldoses and ketoses react with amines in an appropriate solvent to form N-glycosides. Such compounds are very important biologically.
- In the nucleotides and nucleic acids, ring nitrogen atoms of purine or pyrimidine bases form N-glycosidic linkages with carbon atom 1 of D-ribose or 2-deoxy-D-ribose.

3. Sugar phosphates:

- Phosphate derivatives of Monosaccharides are found in all living cells, all reactions in the cells occurred at high energy level that level in carbohydrates reached by phosphorylation by phosphorylase or kinase enzymes to sugar phosphates in which they serve as important intermediates in carbohydrate metabolism.
- The phosphoric acid esters of trioses are glyceraldehyde phosphate and dihydroxyacetone phosphate.

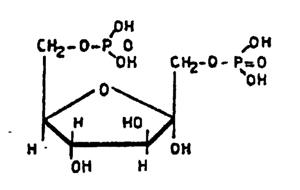
O
||
C-H
|
H-C-OH
|
CH_2-O-P

Glyceraldehyde 3-phosphate

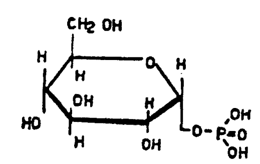
CH_2OH
|
C=O
|
CH_2-O-P

Dihydroxyacetone phosphate

- D-ribose phosphate and D-deoxyribose phosphate are formed in nucleotides and Co-enzymes.
- Glucose phosphate and fructose phosphate are important compounds in many processes as fermentation, absorption and metabolism of carbohydrates.



Fructose-1,6-diphosphate



Glucose-1-phosphate

4. Deoxy sugars <u>(sugars lack an oxygen):</u>

- Deoxy sugars are those in which a hydroxyl group attached to the ring structure has been replaced by hydrogen atom.
- Several deoxy sugars are found in nature. The most abundant is 2-deoxy-D-ribose, the sugar component of deoxyribonucleic acid L-fllcose (6-deoxy L-galactose) is another important deoxy sugar present in some bacterial cell wall.

H-C=O
|
H-C-H
|
H-C-OH
|
H-C-OH
|
CH_2OH

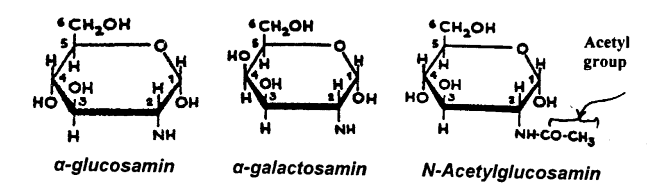
2-Deoxy –D-ribose

H-C=O
|
H-C-OH
|
HO-C-H
|
HO-C-H
|
HO-C-H
|
CH_3

L-fucose (6-deoxy-L-galactose)

5. Amino sugars:

- Sugars containing an amino group are called amino sugars are compounds of glycoproteins, gangliosides and glucosamino-glycans.
- Two amino sugars of wide distribution are D-glucosamine (2-amino-2-deoxy-D-giucose) and D-galactosamine (2-amino-2-deoxy-D-galactose), in which an amino group replaces the hydroxyl group at carbon atom 2.
- Glucosamine is obtained by hydrolysis of polysaccharide chitin.
- It is also found in glycoproteins, mucoitin sulphate, in heparin and in hyaluronic acid.
- Galactosamine is found in chondroitin sulphate, the major polysaccharide of cartilage.
- The amino sugars are usually found in these compounds as N-acetyl derivatives.
- Several antibiotics (erythromycin, carbomycin) contain amino sugars which are believed to be related to the antibiotic activity of these drugs.



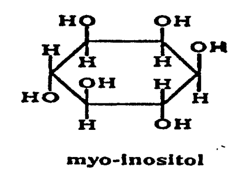
α-glucosamin *α-galactosamin* *N-Acetylglucosamin*

6. Osazones

- As mentioned in chemical reactions of monosaccharides, osazones are used to identify sugars.

7. Sugar alcohols:

- Besides D-sorbitol, D-mannitol, D-dulcitol and D-ribitol produced by reduction of monosaccharides, there are two other sugar alcohols occur in nature:-
- One is glycerol, an important component of some lipids.
- The other is fully hydroxylated cyclohexane derivative inositol, which can exist in several stereoisomeric forms. One of the steroisomers of inositol, moy-inositol is found in the lipid phosphatidylinositol which used as internal signal. Also myo-inositoi is present in phytic acid, the hexaphosphoric ester of inositol which henders the absorption of calcium phosphorus and magnesium.



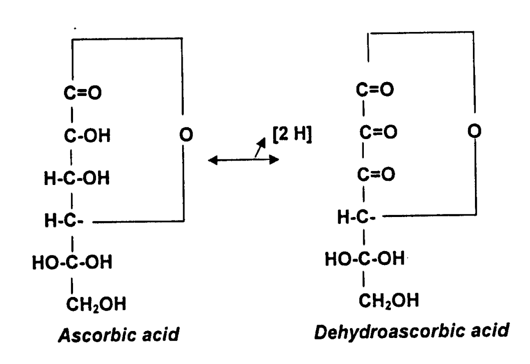
myo-Inositol

8- Sugar acids:

- Besides the three important types of sugar acids : aldonic, aldaric and uronic acids, there is another important sugar acid which is L-ascorbic acid (vitamin C) D-ascorbic acid is not vitamin. Possesses two asymmetric carbon atoms (4 &5). It occurs in L-fonn and has an enediol structure. Reducing characters and acidity are due to this enediol group. It is a very unstable compound and readily undergoes oxidation to dehydroascorbic acid.

- Prolonged lack of ascorbic acid in the diet of human beings results in scurvy.

- Also, relative deficiency of ascorbic acid may produce alterations in connective tissue structure and may also cause decreased resistance to some infections. Ascorbic acid is present in large amounts in citrus fruit and tomatoes.

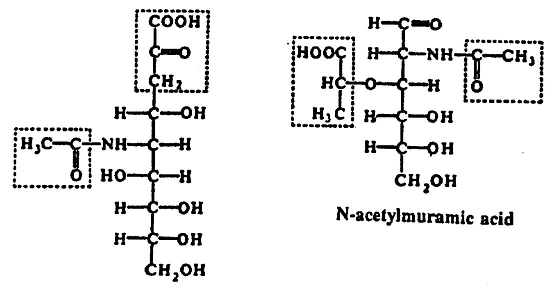


Ascorbic acid ***Dehydroascorbic acid***

9. *Muramic acid and neuraminic acid*

- These sugar derivatives are important building blocks of the structural polysaccharides found in the cell walls of bacteria and the cell coats of higher - animal cells, respectively.
- Both are nine - carbon amino sugar derivatives.
- They may be visualized as consisting of a six - carbon amino sugar linked to a three carbon sugar acid, the amino group is usually acetylated.
- N-acetyl muramic acid consists of N-acetyl D-glucosamine in either linkage with the three-carbon D-lactic acid.
- N-acetyl neuraminic acid is derived from N-acetyl D-matnnosamine and pyruvic acid.
- It is an important building block of the oligo-saccharide chain found in the glycoproteins and glycolipids of the cell coats and membranes of animal tissues.

N-acetyl derivatives of neuraminic acid are generally called sialic acids. The sialic acid found in the human tissues contains an N-acetyl group.



N-acetylmuramic acid

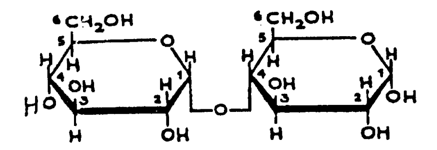
N-acetylneuraminic acid

DISACCHARIDES

Disaccharide consists of two Monosaccharides joined by a glycosidic linkage. Their chemical name reflects their component Monosaccharides. The most common disaccharides are maltose, lactose and sucrose.

Maltose:

- It is called malt sugar due to it is present in barely.
- It is formed as an intermediate product of the action of amylases on starch.
- It contains two D-glucose residues. Both glucose moieties are in α-pyranose form, linked by α1,4 glycosidic linkage.
- The second glucose residue of maltose has a free anomeric carbon atom capable of existing in α and β forms. The first glucose residue can not undergo oxidation, but the second residue can. It is called the reducing end. Maltose forms osazone, shows mutarotation and is fermented by yeast.

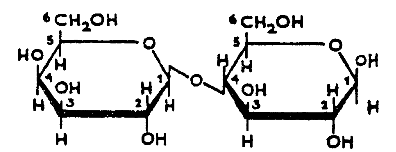


O-α-D-Glucopyranosyl-(1 → 4)-α-D-glucopyranoside

Lactose:

- The disaccharide lactose is found in milk but otherwise does not occur in nature It yields D-galactose and D-glucose on hydrolysis.

- Since it has free anomeric carbon on the glucose residue, lactose is a reducing. disaccharide. It is not sweet 1/5 of home sugar to enable the baby to drink much milk.
- Lactose forms osazone and shows mutarotation. It is not fermented by yeast. Lactose is hydrolysed by acids or enzyme lactase in the intestine into glucose and galactose.



O-β-D-Galactopyranosyl-(1 → 4)-β-D-glucopyranoside

Sucrose:

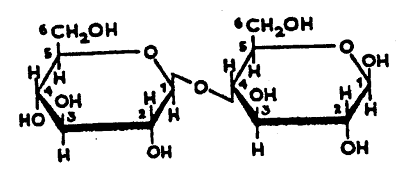
- Sucrose or cane sugar is a disaccharide of glucose and fructose. It is extremely abundant in the plant world and is familiar as table sugar or home sugar.
- Unlike most disaccharides and oligosaccharides, sucrose contains no free anomeric carbon atom.
- The anomeric carbon atom of the two hexoses are linked to each other. For this reason sucrose does not undergo mutarotion, does not react with phenylhydrasine to form osazone and does not act as a reducing sugar.
- It is much more readily hydrolyzed than other disaccharides.
- The hydrolysis of sucrose to D-glucose and D-fructose is often called inversion since it is accompanied by a net change in optical rotation from dextro to levo as the equimolar mixture is called invert sugar.

SUCROSE

O-β-D-Fructofuranosyl-(2 → 1) –α-D-glucopyranoside

Cellobiose:

- It consists of two β-D-glucose units linked by β (1 → 4) glycosidic linkage. It represents the repeating disaccharide unit of cellulose. It is a reducing sugar since it has a free anomeric carbon atom.



O-β-D-Glucopyranosyl-(1 → 4)-β-D-glucopyranoside

Gentibiose:

- It contains of two β-D-glucose units linked by β (1 → 6) glycosidic linkage. It shows reducing properties since it has free anomeric carbon atom. It founds in the germinated seeds and legumes.

O-β-D-Glucopyranosyl-(1 → 6)-β-D-glucopyranoside

Trehalose:

- It contains two α-D-glucose residues linked by α (1→ 1) glycosidic linkage, so it is non-reducing disaccharide in which the two anoineric carbon atoms are joined.

It is the major sugar found in haemolymph of many insects.

O-α-D-Glucopyranosyl-(1 → 1)-α-D-glucopyranoside

POLYSACCHARIDES

Most of the carbohydrates found in nature occur as polysaceharides of high molecular weight. On complete hydrolysis with acid or specific enzymes, these polysaccharides yield Monosaccharides and /or simple monosaccharid derivatives.

General characters of polysaccharides:

1. They are powder in nature.
2. They are insoluble in water and form colloids.
3. They are not optically active.
4. They do not show mutarotation.
5. They do not form osazone.
6. They are not reducing sugars.

Polysaccharides, which are also called glycans differ in the nature of their recurring monosaccharide units, in the length of their chains and in the degree of branching.

Classification of polysaccharides

I. Chemical classification

1. Simple (homopolysaccharides):

On hydrolysis they yield the same units of monosaccharides or monosaccharide derivative.

a) Pentosans *e.g.* Xylans.

b) Hexosans:

1- Glucosans *e.g.* *starch, glycogen, celluose.*
2- Mannosans *e.g.* *yeast wall.*
3- Fructosans *e.g.* *Inulin.*
4- Galactosan *e.g.* *agar agar.*
5- Glucosaminan *e.g.* *chitin.*
6- Galacturonican *e.g.* *pectin.*

2. Mixed (heteropolysaccharides):

On hydrolysis they yield more than one type of monosaccharides.

a) Acidic (Mucopolysaccharides)

i- Sulfate free acidic heteropolysaccharides (amino sugar + uronic acid): *e.g.*

* Hyaluronic acid.
* Prosthetic group of FSH.
* Human chorionic gonadotrophin.

ii- Sulfated acidic heteropolysaccharides (amino sugar + uronic acid + H_2SO_4): e.g.

* Mucoitin sulphate.
* Chondroition sulphate.
* Heparin.

b) Neutral (mucoprotein):

Amino sugars with no uronic acid or H_2SO_4 : e.g.

* α 1 and α 2 globulins.
* Blood groups.
* TSH.

II. Functional classification:

1).Structural (supportive):

e.g. * Cellulose in plants. * Mucopolysaccharides.

2) Nutrient polysaccharides:

*e.g.** Starch in plants. * Glycogen in animals.

Starch:

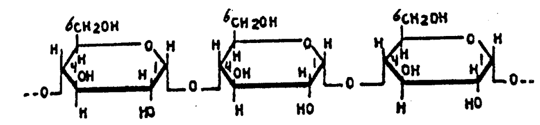
Starch occurs in two forms amylose and amylopectin.

1- Amylose Consists of long unbranched chains in which all the D-glucose units are bound in α (1 → 4) linkages. The chains vary in molecular weight from a few thousands to 500,000.

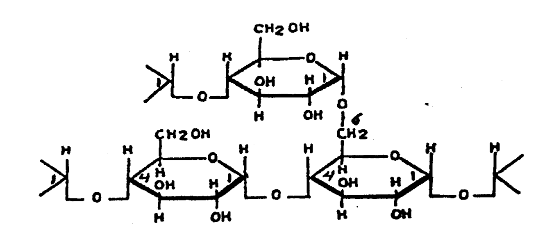
Amylose gives blue colour with iodine. It is found in the nucleus of starch granule.

2- Amylopectin is highly branched, the average length of the branches is from 24 to 30 glucose residues, depending on the species. the backbone glycosidic linkage is α (l → 4) but the branch bands are α (l → 6) linkage.

- Amylopectin gives a red-violet colour with iodine.
- Its molecular weight may be as high as 100 million.
- It forms the shells of starch granules.



Amylose [α-glucose units linked together by α(1-->4) glycosidic linkages]



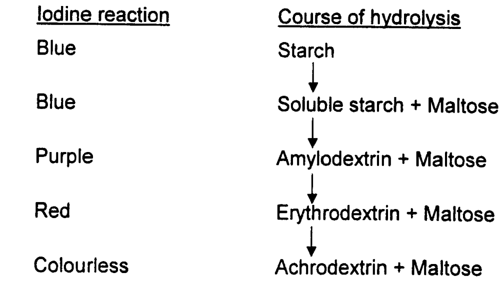
Amylopectin [α-glucose units linked together by α(1-->4) and at branch points by α(1-->6) glycosidic linkages] ly

- Amylose can be hydrolyzed by α amylase, which is present in saliva and pancreatic juice and participates in the digestion of starch in the gastrointestinal tract. It hydrolyzes α(1 → 4)

linkages at random to yield a mixture of glucose and free maltose, the latter is not attacked.

- Amylose can also be hydrolyzed by *B*-amylase. This enzyme which occurs in malt, cleaves away successive maltose units beginning from the non-reducing end to yield maltose quantitatively.
- The polysaccharides of intermediate chain length that are formed from starch components by the action of amylases are called <u>dextrins</u>. Neither α nor B- amylases can hydrolyze the linkage at the branch points of amylopectin (1 → 6 glucosidic linkage).
- The end product of B-amylase action on amylopectin is a large, highly branched core, or limit dextrin.

Action of â-amylase on starch

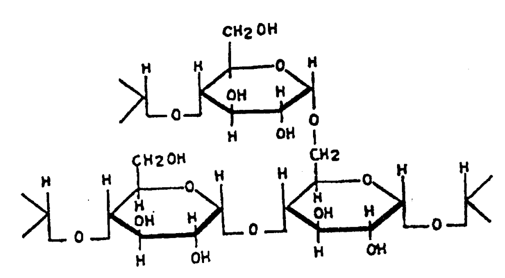


Iodine reaction	Course of hydrolysis
Blue	Starch
	↓
Blue	Soluble starch + Maltose
	↓
Purple	Amylodextrin + Maltose
	↓
Red	Erythrodextrin + Maltose
	↓
Colourless	Achrodextrin + Maltose

Glycogen:

- Glycogen is the main storage polysaccharide of animal cells. Glycogen is abundant in the liver and muscles. Sometimes called animal starch.
- Like amylopectin, glycogen is a polysaccharide of D-glucose residues in α (1 → 4) linkages.

- However, it is a more highly branched and more compact molecule than amylopectin. The branches occur about every 8 to 12 glucose residues.
- The branch linkages are α (1 → 6). Glycogen is readily hydrolyzed by α and B-amylases to yield glucose and maltose respectively; the action of â-amylase also yields a limit dextrin. Glycogen gives a red - violet colour with iodine.



Glycogen [α-glucose units linked together by α(1-->4) and at branch points by α(1-->6) glycosidic linkages]

Dextrans:

- These are polysaccharides produced by certain bacteria when allowed to grow on sugar media.

- They are branched polysaccharides of D-glucose residue but they differ from glycogen and starch in having backbone linkages other than α (1→ 4) found as storage polysaccharides in yeast and bacteria, they vary in the branch points, which may be I → 2, 1 → 3, 1 → 4 or I → 6 in different species. Dextrans form highly viscous, slimy solutions.

Inulin:

— It is a polysaccharide found in plants e.g. in artichoke.

— It consists of D-fructose residues *(about 30 units)* in α (2 → 1) linkage.

It is an inert substance – not digested – not reabsorbed so. It was used kidney function tests.

Chitin:

— It is homopolysaccharide consists of N-acetyl-glucosamine residues joined by β (1 → 4) linkage. Chitin is present in shells of lobsters and covering coat of insects.

Agar agar:

— It is found m seaweeds and is used in the preparation of culture media in bacteriology. It is formed of galactose units esterifled with sulphuric acid.

Pectins:

— They are found in plants especially in fruits specially the outer layer of water millions and are responsible for the setting of jam.

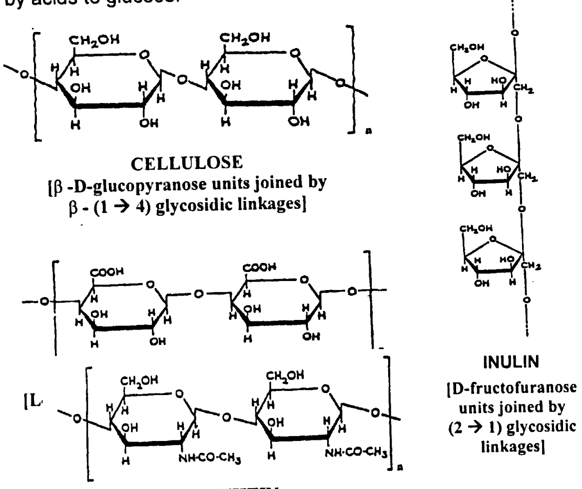
— Pectin is formed of long chain of galacturonis acid units joined by β (l → 4) glycosideic linkage.

Cellulose:

- This is the most important of the structural polysaccharides in plant.
- On complete hydrolysis of cellulose, D-glucose is formed while partial hydrolysis gives the disaccharide cellobiose. Cellulose consists of a straight chain of D-glucopyranose units joined by β (l → 4) glucosidic linkages.
- It is found in wood, cotton (nearly pure cellulose) and in other plants. Cellulose is not digested in our body. Bacteria, which

are, present in the alimentary canal of some animals (ruminants) help them to digest cellulose, by their enzymes cellulases.

- Cellulose is of value to human being in the fact that it increases the intestinal contents and its bulk stimulates peristalsis and elimination of food residues
- Cellulose is insoluble in water and not reducing. It is hydrolyzed by acids to glucose.



CELLULOSE
[β -D-glucopyranose units joined by β - (1 → 4) glycosidic linkages]

INULIN
[D-fructofuranose units joined by (2 → 1) glycosidic linkages]

[L

CHITIN
[N-acetyl-D-glucosamine units joined by â- (1 → 4) glycosidic linkages]

Mucopolysaccharides (Glycosaminoglycans):

- These are acidic substances which contain uronic acids and N-acetylated amino sugars which may be sulfated.
- The sugar residues form molecules of high molecular weight, which combine with protein.
- The carbohydrate part is easily separated from the protein.

Classified into non-nitrogenous and nitrogenous:

1- ***Non-nitrogenous*** mucopolysaccharides consist of Monosaccharides and uronic acids.

I- Hemicellulose:

Which is formed from hexoses, pentoses and uronic acid it is found a long with legnin in plants.

II- Plant gum: has nearly the same composition.

2- ***Nitrogenous mucopolysaccharides***

I- Neutral:

A- Glycoprotein *(mucoprotein)* carbohydrates + protein.

Example:

Mucin of saliva, gonadotrophic hormones *(FSH, LH and HCG).*

B- Blood group contain *[galactose, galactosamine, fucose and glucosamine]*

II- Acidic:

1) Sulphate-free: *example*: Hyalouronic acid.
2) Sulphated: *example*: Mucoitin sulphate – chonderiotin sulphats and Heparin

1- Sulphate-free:

Hyaluronic acid:

Structure

— It is formed of repeated units of β-glucuronic acid and N-acetyl glucosainine linked by β (I → 3) glycosidic linkage. The repeated units are joined by β (I →4) linkage.

β-Glucuronic acid N-Acetylglucosamine

HYALURONIC ACID

Distribution

* Vitreous body of the eye.
* Synovial fluid of joints.
* Cementing substance in the subcutaneous tissue.

An enzyme, hyaluronidase, which is found in bacteria and in some snake poisons hydrolyzes hyaluronic acid and thus destroys the strength of the connective tissues and helps the spreading of bacteria in the tissues by increasing their permeability.

Mucoitin sulphate:

— It is present in gastric mucosa.

— It is formed of units of â-glucuronic acid and N-acetyl glucosamine + sulfuric acid linked together by α (I → 3) and in between units by α (I → 4) glycosidic linkage.

β-Glucuronic acid N-Acetylglucosamine

Mucoitin sulphate

Chondroitine sulfate:

It is found the cartilage, skin, tendons, and heart valves.

– On hydrolysis it gives â-glucuronic acid, N-acetylgalactosarnine + sulfuric acid linked together by â (1 → 3) and in between units by â (1 → 4) glycosidic linkage.

β-Glucuronic acid N-Acetylgalactosamine sulfate

CHONDROITIN-4-SULFATE
[Note: There is also a 6-sulfate]

Heparin

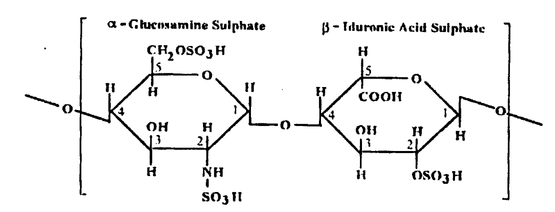
– This is a mucopolysaccharide formed in the liver, spleen, lung and mast cells.

– It inhibits blood coagulation (anti-coagulant).

— The hydrolysis of heparin gives glucuronic acid, N-acetyl-glucosamine and sulfuric add united by α (1 → 4) glycosidic linkage.

— Hydroxyl groups link the sulfuric acid not only to the sugar but also to the amino group of glucosamine units.

— Heparin is probably present in the form of a carbohydrate complex.



HEPARIN

- About 90% of the uronic acid residues are iduronic acid and only 10% are glucuronic acid.
- Heparin has a low molecular weight of about 5000-20'000.
- Heparin activates lipoprotein lipase enzyme.
- Heparin acts as a powerful anticoagulant.

Heparan sulfate:

- It is an extracellular proteoglycan present on cell surfaces. It resembles the intracellular heparin in its disaccharide units but it contains fewer N-sulfates on glucosamine on the hand. On the other hand, glucuronic acid is the predominant uronic acid.

Dermatan sulfate:

- It contains two types of repeating disaccharide units. Glucuronic acid linked by α-1-3 glucosidic linkage to α-aceyl glucosamine and iduronic acid linked to α-N-acetyl glucosamine by α-1-3 glucosidic linkages.
- Dermatan sulfate is widely distributed in animal tissue resembling chondroitin sulfate and heparin sulfate.
- It acts as anti-thrombotic agent similar to heparin.

ii- Glycoproteins:

- Glycoproteins have a molecular weight of 15'000-1'000'000 and their carbohydrate content ranges from 1-85% by weight. Most of the membrane proteins and secreted proteins are glycoproteins. They are neutral. Some fractions of different glycoproteins can be seen in the following list:

a- *Lubricant and protective glycoproteins:*
- Mucins
- Mucous secretions.

b- *Hormones:*
- Thyrotropin (TSH)
- Chorionic gonadotrophins.

c- *Enzymes:*
- Proteases.
- Nucleases.
- Hydrolases.
- Clotting factors.

d- *Immunologic molecules:*
- Immunoglobulins.
- Complement.
- Blood groups.
- Interferone.
- Histocompatibility antigens (HLA typing).

e- *Structural molecules:*
- Cell walls.
- Collagen, elastin.
- Fibrins.
- Bone matrix.

Glycoprotein contain 9 different types of sugar residues which are:

- Glucose.
- Mannose.
- N-acetyl glactosamine.
- Arabinose.
- Sialic acid.
- Galactose.
- N-acetyl glucosamine.
- Frucose (6-deoxy-galactose).
- Xylose.

Lignin:

- This compound is not carbohydrate, but because of its close association with cellulose it is discussed with carbohydrates.
- It gives additional protection to plants.
- It consists of an aromatic nucleus with hydroxyl and methoxyle groups.
- It contains nitrogen varies between 1 to 5 % in different products.
- It is particularly resistant to ruminants and non-ruminants digestion.
- It is used as an indicator for digestibility of other feed staffs.

Saccharin:

- It is a colourless, crystalline substance sparingly soluble in water.
- It is 500 times as sweet as sucrose.
- It is used in it's sodium salt in diabetic patients to replace sugars (diet sweet).

CH_3 $\xrightarrow[-NH_3]{Amination}$ CH_3 $\xrightarrow[KMNO_4]{Oxidation}$ $COOH$

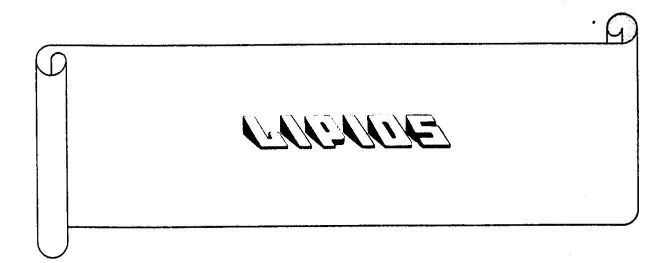
SO_3Cl — SO_2NH_2 — SO_2NH_2

O-Toluen sulphonyl chloride — O-Toluen sulphonamide — O-Benzoic sulphonamid

↓ Heating

O
C
NH
SO_2

Saccharine


LIPIDS

LIPIDS

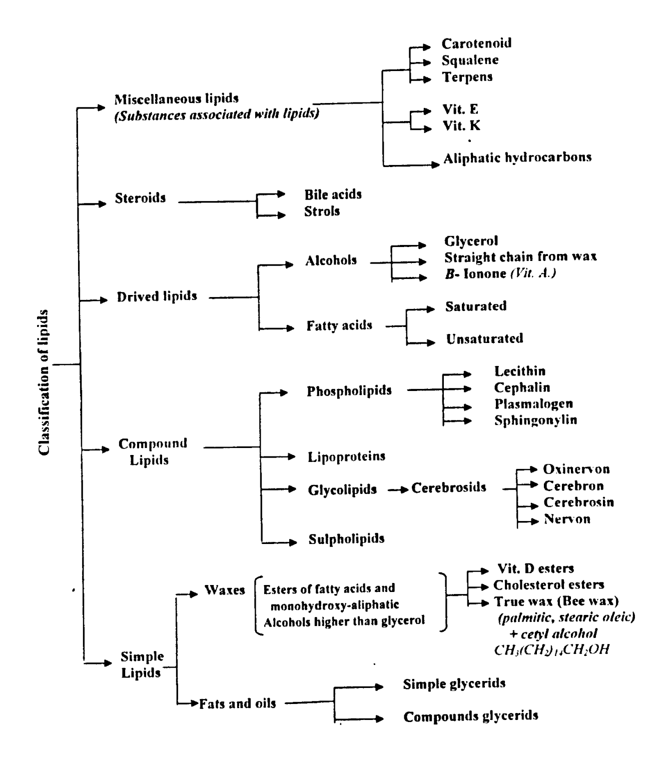
- Heterogeneous groups of compounds related actually or potentially to fatty acids and are formed mainly from alcohol and fatty acids.
- They composed of oxygen, hydrogen, carbon, with nitrogen and phosphate.
- Soluble in non-polar compounds (solvents) as ether, benzyne, .. etc.
- They can be utilized by the living organisms.
- They are important constituent of animals and some plant tissues.
- They are a ready source of energy because they supply over half of energy used in basal metabolism.

  N.B. : Energy k Cal /g *(Human oxidation)*

Protein	= *4.10*
Fat	= *9.30*
Carbohydrates	= *4.10*

- They acted as thermal blankets because their presence in s/c insolate the against heat loss.
- Serve as a thermal insulator in the subcutaneous tissues and also acts as an electrical insulator allowing rapid propagation of depolarization waves along myelinated nerves.
- As a cell surface component, they are concerned with cell recognition, species specificity and tissue immunity.
- Combinations of fat and protein (lipoproteins) are important cellular constituents, occurring both in the cell membrane and in the mitochondria within the cytoplasm. Lipoproteins serve as the means of transporting lipids in the blood.

- Knowledge of lipid biochemistry is important in understanding many areas of interest like obesity, atherosclerosis, and the role of various polyunsaturated fatty acids in nutrition and health.
- They acted as protection cushions for fixation internal organs
- They are integral part of cell membrane.
- They supplied the body with; essential fatty acids, fat soluble vitamins, phosphates in the form of phospholipids
- Due to their presence s/c they give the soothe contour of the body.
- They acted as structures of the secondary sexual characters.
- Cholesterol is used for synthesis of adrenal cortical hormones, Vit. D and bile acids.
- They provide milk with it's fat content.
- The compound lipids are important for brain activities.


Classification of lipids
Miscellaneous lipids
(Substances associated with lipids)
Carotenoid
Squalene
Terpens
Vit. E
Vit. K
Aliphatic hydrocarbons
Steroids
Bile acids
Strols
Drived lipids
Alcohols
Glycerol
Straight chain from wax
B- Ionone (Vit. A.)
Fatty acids
Saturated
Unsaturated
Compound Lipids
Phospholipids
Lecithin
Cephalin
Plasmalogen
Sphingonylin
Lipoproteins
Glycolipids
Cerebrosids
Oxinervon
Cerebron
Cerebrosin
Nervon
Sulpholipids
Simple Lipids
Waxes
Esters of fatty acids and monohydroxy-aliphatic Alcohols higher than glycerol
Vit. D esters
Cholesterol esters
True wax (Bee wax)
(palmitic, stearic oleic)
+ cetyl alcohol
$CH_3(CH_2)_{14}CH_2OH$
Fats and oils
Simple glycerids
Compounds glycerids

FATTY ACIDS

They are compounds represented by the chemical formula ***R-COOH***, where ***R*** stands for an alkyl chain composed of carbon and hydrogen atoms. Fatty acids that occur in natural fats usually contain an even number of carbon atoms and of straight chain. The chain may be saturated (containing no double bonds) or unsaturated (containing one or more double bonds). Also it may be short or long chain. Short chain fatty acids contain 2-10 carbon atoms, while long chain fatty acids contain more than 10 carbons.

The carbon atoms of a fatty acid are numbered either from the carboxyl group or from the carbon atom furthest to the carboxyl group. The a-carbon is adjacent to the COOH and the β- is following it.. etc.

Methyl Terminus →	CH_3-	CH_2-	CH_2-	CH_2-	CH_2-	CH_2-	CH_2-	CH_2-	CH_2-	COOH	← Carbon Terminus
Carbon No.	10	9	8	7	6	5	4	3	2	1	
Omega No.	1	2	3	4	5	6	7	8	9	10	
Letter designation .... **(Greek letters)**					ε	δ	γ	β	α		

Saturated fatty acids:

They have no double bonds. They are based on acetic acid as the first member of the series. Examples of the acids in this series are shown in the following table:

Examples of saturated fatty acid:

Common name	***General formula***
Acetic	$CH_3\text{-}COOH$
Propionic	$CH_3\text{-}CH_2\text{-}COOH$
Butyric	$CH_3\text{-}CH_2\text{-}CH_2\text{-}COOH$
Valeric	$CH_3\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}COOH$
Caproic	$CH_3\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}COOH$
Isovaleric	$CH_3\text{-}\underset{\displaystyle CH_3}{\overset{}{CH}}\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}COOH$
Caprylic	$CH_3.(CH_2)_6.COOH$
Capric	$CH_3.(CH_2)_8.COOH$
Lauric	$CH_3.(CH_2)_{10}.COOH$
Myristic	$CH_3.(CH_2)_{12}.COOH$
Palmitic	$CH_3.(CH_2)_{14}.COOH$
Stearic	$CH_3.(CH_2)_{16}.COOH$
Arachidic	$CH_3.(CH_2)_{18}.COOH$
Behenic	$CH_3.(CH_2)_{20}.COOH$
Lignoceric	$CH_3.(CH_2)_{22}.COOH$

Unsaturated fatty acids:

They contain one or more double bonds. They may be further subdivided according to degree of unsaturation:

A- Monounsaturated (*Monoenoic or monoethenoid*) acids.:

B- Polyunsaturated (*Polyenoic or polyethenoid*) acids.

C- Eicosanoids: These compounds are derived from 20 carbon polyenoic fatty acid. They are precursors to prostaglandins, and triromboxanes, which are biologically active compounds.

Naturally occurring unsaturated fatty acids in mammals are all of the cis configuration. Trans fatty acids are rarely present in the body.

Cis

R   COOH
C = C
H   H

Trans

H   COOH
C = C
H   H

Examples of unsaturated fatty acids:

No. of C atoms & No. position of double bond	*Name*	*Formula*
16 : 1 ; 9	Palmitolec	CH_3-$(CH_2)_5$-CH=CH-$(CH_2)_7$COOH
18 : 1 ; 9	Oleic	CH_3-$(CH_2)_7$-CH=CH-$(CH_2)_7$COOH
24 : 1 ; 9	Nervonic	CH_3-$(CH_2)_7$-CH=CH-$(CH_2)_{13}$COOH
18 : 2 ; 9, 12	Linoleic	CH_3-$(CH_2)_3$-(CH_2-CH=CH$)_2$-$(CH_2)_7$COOH
18: 3; 9,12,15	Linolenic	CH_3-(CH_2-CH=CH$)_3$-$(CH_2)_7$COOH
20:4;5,8,11,14	Arachidonic	CH_3-$(CH_2)_3$-(CH_2-CH=CH$)_4$-$(CH_2)_3$COOH

Hydroxy fatty acids:

- They contain hydroxyl group.
  e.g. - Ricinoleic acid - Dihydroxy stearic acid.
- They found in caster oil have a drastic purgative action.

Cyclic fatty acids:

- Chaulmoogric acid present in chaulnoorgra oil.
- Used as a medicament for the treatment of leprosy.

CH
CH   CH-$(CH_2)_2$-COOH
CH_2 —— CH_2

Prostaglandins (PG):

- They are fatty acid derivatives with hormone like action.
- Prostaglandins are derived from the hypothetical prostanoic acid which contain 20 carbon atoms arranged in 5 membered ring and 2 aliphatic side chains.

Prostanoic Acid

- According to the distribution of OH and C = O (ketonic) groups around the five membered ring and the position of the double bond in the ring, prostaglandins are divided into PGA, PGB, PGE and PGF.
- All prostaglandins are divided into two types according to the number of the double bond in the side chains.
  - Type 1: Contains one double bond (C_{13}-C_{14}) and OH group at C_{15} i.e. PGA_1, PGB_1, PGE_1 and $PGF_{1}\alpha$.
  - Type 2: Contains two double bond (C_5-C_6 & C_{13}-C_{14}) and OH group at C_{15} i.e. PGA_2, PGB_2, PGE_2 and $PGF_{2}\alpha$.
- PGA contains ketonic group at C_9 and double bond at C_{10}-C_{11}.
- PGE contains ketonic group at C_9 and OH group at C_{11}.
- PGF contains two OH groups, one at C_9 and the other at C_{11}.

Biosynthesis of prostaglandins:

- With the exception of RBCs, PGs are produced and released by nearly all mammalian cells and tissues.
- The immediate precursor of PG is arachidonic acid (20:4) which is derived from:
  a- Linoleic acid (18:2) present in diet.

Prostaglandin A_1

[PGA_1]

Prostaglandin A_2

[PGA_2]

Prostaglandin B_1

[PGB_1]

Prostaglandin B_2

[PGB_2]

Prostaglandin E_1

[PGE_1]

Prostaglandin E_2

[PGE_2]

Prostaglandin $F_{1\alpha}$

[$PGF_{1\alpha}$]

Prostaglandin $F_{2\alpha}$

[$PGF_{2\alpha}$]

b- Phospholipids present in all cell membrane by the action of phospholipase A_2.

- The first step in PG synthesis is the oxidation and cyclization of arachidonic acid to give PG_2 and PGH_2 this is done by prostaglandin endoperoxidase synthetase enzyme complex which posses two catalytic activities.

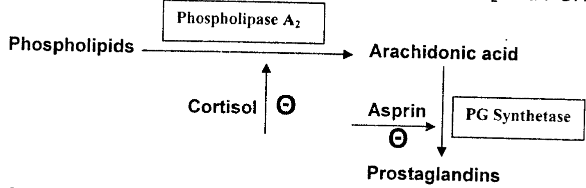
  a- Fatty acid cyclo-oxygenase which requires two molecules of O_2.

  b- Peroxidase which is dependent on reduced glutathione.

- PGH_2 is the precursor for a number of prostaglandins.

Inhibition of PG biosynthesis:

- Cortisol inhibits phospholipase A_2 and thus the precursor of PG (arachidonic acid) is not available.
- Aspirin, indomethacin and phenylbutazone inhibit PG synthetase enzyme, so inhibit the formation of PG_2 and PGH_2.



Catabolism of prostaglandins:

- PGs are not stored in tisues but once synthesized, they are released.
- PGs have a very short half-life, soon after release, they are rapidly taken up by cells and inactivated.
- Inactivation of prostaglandins occurs in the liver by PG 15-hydroxy dehydrogenase enzyme.
- Lungs appear to play an important role in the inactivation of PG.

Physiological effects of prostaglandins:

[1] Inflammation:

PGs appear to be one of the natural mediators of inflammation. Anti-inflammatory drugs such as aspirin and indomethacin inhibit PG synthesis.

[2] Reproduction:

$PGF_{2\alpha}$ induce labour.

PGE play some role in infertility in males.

[3] Gastric secretion and peptic ulcer:

PGs inhibit gastric acid secretion in patients with peptic ulcers. This is due to inhibition of cAMP formation in gastric mucosal cells. PG also accelerate the healing of gastric ulcers.

[4] Regulation of blood pressure:

PGE and PGA decrease systemic arterial blood pressure due to vasodilatation.

[5] Platelet aggregation and thrombosis:

PGI_2 inhibits platelet aggregation.

PGE_2 and thromboxan A_2 (TXA_2) stimulate platelet aggregation.

Essential fatty acids:

Mammals cannot synthesize all of the necessary types of polyenoic fatty acids. Those polyenoic tatty acids must be obtained from the diet. They are termed essential fatty acids.

Importance of essential fatty acids:

- Form part of various membranes.
- Inter in the phospholipid formation.
- Protect the body against X rays of sun.
- Source of certain important materials as prostoglandins.
- Play a part in lipid transport and certain lipoprotein enzymes.
- Transfer to cholesterol

Properties of fatty acids in the body:

- **The physical properties** of body lipids depend on the lengths of carbon chains and degree of unsaturation of their constituent fatty acids.
- The melting point of even-numbered carbon fatty acids increases with chain length and decreases according to unsaturation. A triacylglycerol containing 3 saturated fatty acids of 12°C or more is solid at body temperature whereas it all 3 fatty acids are 18:2 it is liquid to below 0°C.
- Membrane lipids must be liquid, whereas tissue lipids are solid to be used as storage lipids.

Chemical Properties of fatty acids:

Fatty acids present in nature have the following properties:

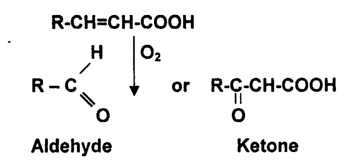
1. They are present in straight chains.
2. They contain even number of carbon atoms.
3. Solubility: This depends on the number of carbon atoms of the fatty acid.
   - If the fatty acid contains from 2-6 carbon atoms, it is soluble in water.
   - If more than 6 carbon atoms, it is insoluble in water and soluble in fat solvents e.g. ether.
   - The Na or K salts of fatty acids (soaps) are soluble in water.
4. Melting point: Saturated fatty acids are solid at room temperature.

   - Unsaturated fatty acids are liquid at room temperature (have a lower melting point).
5. Distillation with steam:
   - Short chain fatty acids (from $C_2 - C_6$) can be distilled with steam (volatile).
   - Long chain fatty acids cannot be distilled with steam (non-volatile).

6. They can from esters with alcohols.
7. Hydrogenation or halogenation: This is a property of unsaturated fatty acids. The hydrogen or halogen is added through the double bond.

$$R\text{-}CH{=}CH\text{-}COOH + H_2 \text{ or } Cl_2$$
$$\downarrow$$
$$R\text{-}CH_2\text{-}CH_2\text{-}COOH$$
$$\text{or}$$
$$R\text{-}\underset{Cl}{\underset{|}{C}}H\text{-}\underset{Cl}{\underset{|}{C}}H\text{-}COOH$$

8. Oxidation: This is another property of unsaturated fatty acids. Oxidation occurs at the double bond producing aldehydes or ketones.



SIMPLE LIPIDS

Fatty acid esters of glycerol = neutral fats. The fatty acid moiety in lipid esters is known as an acyl group. The class of glyceride depends on the number of glycerol alcohol groups that are esterified.

Three general types of glycerides occur:

1. Monoacylglycerols (Monoglycerides):

The single acyl group may be linked to either the primary or secondary alcohol groups i.e., 2 forms of monoacylglycerol are present.

```
        O
        ||
CH2-O-C-R
|
HOCH
|
CH2-OH
```

α-monoglyceride

```
O      CH2-OH
||     |
R-C-O-CH
       |
       CH2OH
```

β-monoglyceride

Monoglycerides are important in digestion and as a metabolic intermediate of lipids.

2. Diacylglycerols (diglycerides):

They contain two acyl groups two types of diglycerides also occur, depending on whether the acyl groups attached to the 1, 2 or 1, 3 alcohol groups of glycerol. diglycerides occur almost as metabolic intermediates in man.

```
                 O
                 ||
O       CH2-O-C-R1
||      |
R2-C-O-CH
        |
        CH2OH
```

1,2 Diacylglycerol

```
        O
        ||
CH2-O-C-R1
|
HOCH    O
|       ||
CH2-O-C-R2
```

1,3 Diacylglycerol

3. Triacylglycerols (triglycerides) = TG:

The 3 alcoholic groups of glycerol are esterified by fatty acids: They are quantitatively the major storage and transport form of fatty adds. The fatty acids may occur in TG in many combinations, e.g. palmitic + 2 oleic, 2 palmitic + linolenic, palmitic + stearic + oleic.... etc.

TG containing fatty acids of the same type e.g. tri-palmitin or tri-stearin is rare to occur.

```
                         O
                         ||
O         1CH2 - O - C - R1
||          |
R2-C-O-2CH           O
            |           ||
          3CH2 - O - C - R3
```

Triglyceride

Physical properties of triglycerides:

1. They are insoluble in water, but soluble in fat solvents e.g. ether, chloroform, benzene, petroleum ether, hot ethyl alcohol and hot acetone.
2. Pure fats are tasteless, odorless, colorless and neutral in reaction.
3. They have low melting points especially if contain unsaturated and short chain fatty acids The liquid fats at room temperature are known as "oils". If TG contains long chain saturated tatty adds (e.g. palmitic or stearic) they will be hard at room temperature *(ghi or margarine).*
4. The specific gravity of all fats is less than 1.0, so they all float on water.

Chemical properties of triglycerides:

1. Hydrolysis:

The fats are hydrolysed by boiling with acids, alkalis or enzymatically giving rise to glycerol and 3 fatty acids. The hydrolysis by alkali is termed saponification due to the formation of the alkali salt or soap. Soaps are cleaning agents because of their emulsifying action.

$$\begin{array}{l} CH_2\text{-O-C(=O)-R} \\ | \\ \text{R-C(=O)-O-CH} \\ | \\ CH_2\text{O-C(=O)-R} \end{array} + 3\,NaOH \rightarrow \begin{array}{l} CH_2OH \\ | \\ \text{HO-CH} \\ | \\ CH_2OH \end{array} + 3\,RCOO\text{-}Na$$

Fat **Glycerol** **Soap**

Sodium soaps are the hard ordinary soaps, whereas potassium soaps are soft (*shaving cream*) but both of them are

soluble in water. Calcium and magnesium soaps are insoluble. In dilute solutions soaps undergo partial hydrolysis giving rise to fatty acids (weak acid) and sodium hydroxide (strong base) so the medium will be alkaline.

$$R\text{-}COONa + H_2O \longrightarrow R\text{-}COOH + NaOH$$

When it is necessary to isolate the fatty acids or sterols present in a given lipid, the material is treated with an alcoholic potassium hydroxide solution. The ester bonds are saponified (as shown above) leaving the sterols in the mixture that may be extracted by hexane.

Addition reactions: (*hydrogenation and halogenation*)

Some elements e.g. hydrogen and halogens can be added to fat containing unsaturated fatty acids. Hydrogenation of oils (hardening of oils) such as cottonseed oil gives rise to more saturated products e.g. margarine.

Disadvantages of margarine:

1. It dose not contain fat-soluble vitamins (A, D, E and K).
2. It does not contain essential fatty acids.

Under suitable conditions a fat can taken up iodine quantitatively at each double bond.

$$-CH = CH- + I_2 \longrightarrow -\underset{}{\overset{I}{\overset{|}{C}}}H-\overset{I}{\overset{|}{C}}H-$$

3. Oxidation:

I- The unsaturated tatty acids are also oxidisable at the point of unsaturation to form peroxide or ketone derivatives.

$$-CH = CH- \longrightarrow -\underset{O-}{\underset{|}{C}}H-\underset{O}{\underset{|}{C}}H \text{ Peroxide}$$

B- drying of oils:

Oils that contain highly unsaturated fatty acids (linseed oil) are spontaneously oxidized by atmospheric oxygen and form hard water proof material, so they are used for painting.

4. Rancidity:

- It is a chemical change that results in unpleasant odour and taste in fat.
- It is caused by light, heat, moisture or bacterial action.
- The oxygen of air attacks the double bonds in fatty acids to form a peroxide linkage.
- Lead or copper catalyzes rancidity. Rancidity may be hydrolytic or oxidative.
- Hydrolytic rancidity causes production of volatile fatty acids that are of unpleasant odour.
- Oxidative rancidity causes production of peroxides and hydroxy fatty acids.

Effect of rancid fat:

1. The products of rancidity are toxic.
2. Products of rancidity destroy other factors of food e.g. vitamins A and C.

Protection of rancidity:

1. Replacing oxygen by inert gas e.g. N_2.
2. Put foods in vacuum.
3. Exclusion of oxygen or the addition of an antioxidant delays the process. Antioxidant substances in diet are vit A and vit E.

Identification of fats and oils:

The most common constants which are of value in identification of fat include the melting point, refractive index, specific gravity, iodine number, Reichert-Meissl number, acid number, acetyl number, saponification number, and acrolin test.

1. Iodine number:

- It is the number of grams of iodine taken. up by 100 grams of fat.
- It is useful in determining the degree of unsaturation of fat.

2. Saponification number:

- It is the number of milligrams of KOH used up in saponification of one gram of fat.
- Fats containing short chain adds have more carboxyl groups per gram than those of long chain acids and will take more alkali.

3. Reichert-Meissl number:

- It is the number of milliliters of 0.1 Normal KOH required to neutralize the volatile fatty acids distilled from five grams of fat after saponification and acidification.
- It measures the degree of rancidity.

4. Acid number:

- It is the number of milligrams of KOH required to neutralize the free tatty acids found in one gram of fat.
- This is important in detection of rancidity.
- Fresh fats give a low acid number, while rancid fats give a high acid number due to free fatty acids liberated by hydrolysis.

5. Acetyl number:

- It is the number of milligrams of KOH used up to neutralize acetic acid liberated from hydrolysis of one gram of acetylated fat.
- It is used for detection of hydroxy fatty acids (as the acid is attached the OH group) e.g. hydroxy nervonic acid found in caster oil.

6. Polyneske number:

- It is the number of KOH needed for neutralization of insoluble volatile fatty acids present in one gram of fat after saponification, acidification, distillation and ice coiling.

7. Acrolin test:

- It depends on the production of acrolin compound by the reaction of glycerol containing fat with KHSO4 potassium hydrogen sulfate).
- Acroline compound has an irritant odour and converts silver nitrate into metalic silver.

$$H_2C(OH)-HC(OH)-HC(H)-O(H) \xrightarrow[\text{DEHYDRATED WITH HEAT PLUS } KHSO_4]{2H_2O} CH_2=CH-CHO$$

Conversion of glycerol to acrolein.

WAXES

These are esters of higher fatty acids combined with certain alcohols but not glycerol.

Properties:

1. Insoluble in water but soluble in fat solvents.
2. Not easily hydrolyzed.
3. Not digested.

Examples:

1- Bees wax. 2- Spermaceti = Sperm oil.
3- Carnuba wax. 4- Lanolin or wool wax.

1. ***Bees wax:*** It is formed by honey bees and is formed from mericyl alcohol and palmitic acid.
2. ***Spermaceti or Sperm oil:*** It is found in the skull of certain sperm whales. It is formed chiefly by cetyl alchol (contains 16 carbon atoms) and palmitic acid. It is used in the manufacture of candles.
3. ***Carnube wax:*** It is a plant wax derived from leaves.

4. *Lanolein or wool fat:* It is prepared from the fibers of wools. It contains a type of alcohol called cholesterol. It is used in preparation of all ointments.

COMPOUND LIPIDS

They are called compound because they contain besides the alcohol and fatty acids other substances e.g. phosphate group, nitrogenous base, carbohydrate, proteins, sulphur and amino groups.

Types:

1) Phospholipids. 2) Glycolipids.

3) Lipoproteins. 4) Sulpholipids and amino lipids.

The phospholipids include:

A. Glycerophospholipids (Glycerophosphatids)

1- Phosphatidic acid:

It is important as an intermediate in the synthesis of TG and other glyceophosphatides

```
                          O
                          ||
     O       CH2- O -C-R1
     ||       |
R2-C-O-CH        O
              |       ||
             CH2-O-P-OH
                       |
                      OH
```

Phosphatidic acid

2- Phosphatidylcholine (Lecithin):

- It contains glycerol, two fatty acids, phsophoric acid and choline.
- Lecithin's are widely distributed in the cells of the body; have both metabolic and structural (cell membrane) functions.

- Dipalmityl lecithin is a very effective surface-active agent, preventing adherence due to surface tension of the inner surface of the lungs.
- Most phospholipids have a saturated acyl radical in C_1 position but an unsaturated radical in C_2 position.

```
                        O
                        ||
      O       1CH2-O-C-R1
      ||        2|
R2-C-O-CH       O                    CH3
                 |      ||                 +  /
              3CH2-O-P-O-CH2-CH2-N-CH3
                         |                     \
                        OH                     CH3
```

Phosphatidylcholine

Chemical properties:

1. Solublity:
   1- Slightly soluble in water and form colloidal solution.
   2- Soluble in fat solvents except acetone.
   3- When fresh it is yellow solids.
   4- It is oxidized rapidly giving black colour due to oxidation of the unsaturated fatty acids in it.

2- *Hydrolysis:*
   1- By alkalis produce soaps, glycerol, H_3PO_4 and choline.
   2- Enzyme (lecithinase A) to lysolecithin.

3- *Importance:*
   1. Inter in the formation of cell wall and membrane.
   2. Necessary in fat metabolism in liver.

4. Source:
   1. Egg yolk. 2.Brain tissues.

Lecithinase enzyme present in cobra venom splits the fatty acid in C_2 producing Lysolecithin which cause haemolysis of RBCs. Lysolecithin is also an intermediate metabolite.

CHOLINE

Lysolecithin

3. Phosphatidyiethanolamine (*cephalin*):

Its structure is as in lecithin but ethanolamine replaces choline. It is also present in cell membrane, and is an essential factor in blood clotting.

Phosphatidylethanolamine

4. Phosphatidylinositol (*Lipositol*):

- It contains inositol instead of the nitrogenous base (choline or ethanolamine).
- It is an important constituent of the cell membrane.
- On hydrolysis phosphatidylinositol gives rise to one mole of glycerol, one mole of inositol, two moles of fatty acids, and one, two or three moles of phosphoric acid.
- Inositol triphosphate also may be produced which is important as internal signal.
- They are present in brain and muscles.

Phosphatidylinositol

5. Phosphatidylserine:

It contains the amino acid serine instead of choline in lecithin. It is cephalin like and present in tissues.

Phosphatidylserine

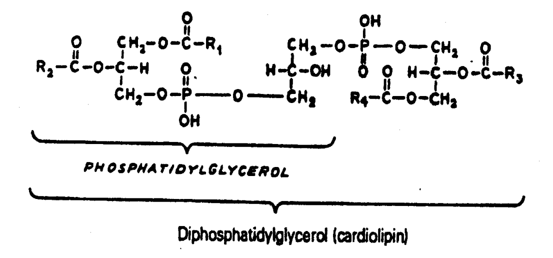
6. Plasmalogens:

- They are present In brain and muscles.
- They resemble the phosphatidylethanolamine but have an ether link on C_1 with unsaturated alcohol instead of normal ester link.
- The alkyl radical is an unsaturated alcohol.
- Choline, serine or inositol may be a base instead of ethanolamin.
- Found in membrane of muscle and nerve cells.
- Present in liver, brain and cardiac and skeletal muscles.

Structure of plasmalogen (phosphatidal ethanolamine)

7. Cardiolipin:

- It is composed of two moles of Phosphatidic acids attached to a glycerol molecule.
- It is present in muscles (especially cardiac muscles) and in cell membrane.
- It is used for test of syphilis.



Diphosphatidylglycerol (cardiolipin)

Properties and biological importance of phospholipids:

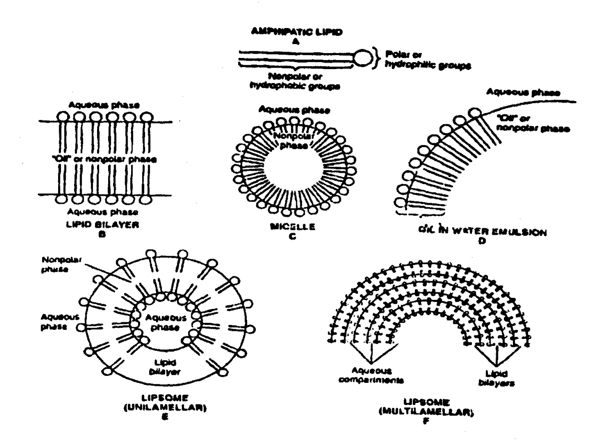
1. They are soluble in fat solvents, but not soluble in acetone. They have a non-polar head (FA) and a polar head (phosphate).
2. They are insoluble in water but they emulsify in it. They have a great affinity for water (hydrophylic), so they function to

transport fatty substances across the intestinal mucosa and through the blood stream.

3. They form Zwitter ion, -ve charge from phosphate and +ve charge on the base.
4. They are widely distributed in mammalian tissues especially the nerve tissues.
5. They are important constituents of the cell membranes, where they influence the transport of substances across them (perform selective permeability).
6. The amount of phospholipids remains constant even during extreme starvation, they are the constant element of fat, while TG is the variable element of. tat.

N.B. When a polar lipid e.g. phosphoglycerides is added to water, it will associate in many aggregates resembling micelles. In such structures, the hydrocarbon tails are hidden from the aqueous medium and form an internal hydrophobic phase; whereas the hydrophilic heads are exposed on the surface, these polar heads will form hydrogen bonds with H_2O.

Dietary lipids as triacylglycerols are non-polar lipids, because of their long hydrocarbon tails, have very little tendency to dissolve in water to give a true solution. However, it readily disperses in water to form micelles. The polar lipid will take up the Non-polar lipids within the interstices of the micelles structure (mixed micelles), so they can disperse these dietary lipids in aqueous material within the micelles, additional attractive forces between adjacent hydrocarbon structures are present.



B- Sphingophospholipids *(Sphlngophosphatldes, Sphinpomyelin):*

Another series of lipids that occur in man are derivatives of sphingosine.

This 18-carbon atom dihydric alcohol contains an amino group at C_{17}.

Long chain fatty acid (usually of 18 carbons or more), is attached to the amino group in amino linkage.

$$\underset{}{CH_3\text{-}(CH_2)_{12}\text{-}CH{=}CH}\text{-}\underset{\large OH}{\underset{|}{CH}}\text{-}\underset{\large NH_2}{\underset{|}{CH}}\text{-}CH_2OH$$

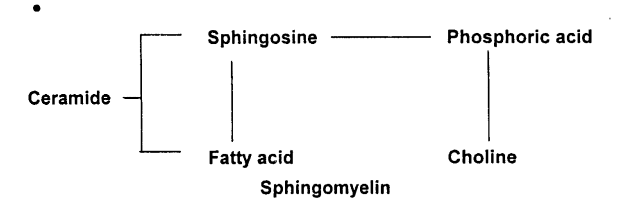
Sphingosine (Sphingol)

- Sphingosine contains an amide linked fatty acid is known as ceramide.
- There are two types of ceramide derivatives in human tissue, sphingomyelins and glycolipids.
- This group of phospholipids is important membrane components in both plant and animal cells.

- They are present in large amounts in brain and nerve tissues.
- Only trace amounts of sphingolipids presented in depot fat.

Sphingomyelins:

- They are found in large quantities in cell membrane. brain and nerve tissue.
- They contain a phosphoryicholine linked to the terminal hydroxyl group of ceramide.
- Sphingomyelins are not soluble in ether but soluble in chloroform, benzene and hot alcohol.
- They are white and crystalline.
- Increased amount of sphingomyelins occurs in liver and spleen in case of Niemann-pick disease (lipidosis).
-



Sphingomyelin

SPHINGOSINE

$$CH_3-(CH_2)_{12}-CH=CH-CH(OH)-CH(CH_2-O-\underbrace{P(=O)(OH)}_{\text{PHOSPHORIC ACID}}-O-\underbrace{CH_2-CH_2-\overset{+}{N}(CH_3)_3}_{\text{(CHOLINE)}})-NH-\underbrace{C(=O)-R}_{\text{FATTY ACID}}$$

Structure of a sphingomyelin

<u>Glycolipid</u> *<u>(Glycosphingolipids)</u>*

- They are ceramides that contain a carbohydrate residue.
- They are widely distributed in every tissue of the body, particularly in nervous tissue and brain.
- They occur in outer leaflet of the plasma membrane where they contribute to cell surface carbyhydrates.

<u>Types of glycoolipids:</u>

According to type of <u>fatty acid</u> and carbohydrate present we have different types of cerebrosides:

1. *<u>Kerasin</u>*: containing lignocertic acid and galactose.
2. *<u>Phrenosine or cerebron</u>*: contains hydroxy lignoceric acid (cerebronic acid) and galactose.
3. *<u>Nervon</u>*: which contains unsaturated lignoceric acid (Nervonic acid) and galactose.
4. *<u>Hydroxy nervon</u>*: contains unsaturated hydroxy lignoceric acid (hydroxy nervonic acid) and galactose.

The previous four previous types of glycolipids are present in the white matter of cerebral hemispheres and in myelin sheaths of nerves.

There are other types of cerebrosides containing other types of carbohydrates e.g.

1- <u>Cytolipin H</u>:

- Contains lactose instead of galactose.
- It is present in high concentration in the spleen in certain disease called Gaucher's disease. In this disease, the cerebroside may contain glucose, galactose or lactose. Cytolipin H is also present in certain tumours and in RBCs.

2- *Globosides:*

They are cerebrosides containing acetylated amino sugars in addition to other hexoses *e.g.* glucose & galactose.

The amino sugar usually present is galactosamine. The chemical structure of globosides can be simply illustrated as follows: ceramide – glucose – $(\text{galactose})_2$ – N-acetyl galactosamide.

It is usually present in the spleen, liver and red cells.

3- *Hematosides*:

This is another type of cerebrosides containing glucose, galactose and sialic acid together with the ceramide.

The sialic acid present is usually N-acetyl neuraminic acid. Ceramide – (glucose) – (galactose) – (sialic acid). They are usually present in red cells, in brain and spleen.

Properties:

1. Glycosphingolipids are almost insoluble in ether but more soluble in acetone than the Glycerophospholipids. They are also soluble in benzene, chloroform and hot alcohol.
2. Neutral glycosphingolipids are constituents of the outer plasma cell membrane (cell surface component). Glycosphingolipids may be important in intercellular communication and contact. Some are antigens and ABO blood group substances.
3. Cancer cells have specific glycosphingolipids other than normal cells.

– In Gaucherie's disease, there is an abnormal large amount of cerebrosides in the liver and spleen *(lipidosis).*

– We can identify hydroxy containing fatty acids by Acetyl Number.

Galactosylceramide (Cerebroside):

They are ceramides that contain a D-galactose residue at the terminal hydroxyl group.

The galactose is linked to the hydroxyl through a α-glucosidic bond.

Galactoceramides do not contain phosphorus and are therefore not phospholipids.

These glycolipids are present in the brain and peripheral nervous system.

SPHINGOSINE

$CH_3-(CH_2)_{12}-CH=CH-CH(OH)-CH-NH-C(=O)-R$

FATTY ACID

GALACTOSE

Structure of a cerebroside

Sulfatide *(Sulfolipids):*

- It is formed when a sulfate group is attached to the hydroxyl group of galactose in galactosylceramide.
- So they are sulphate esters of galactocerebrosides.
- They are present in brain tissues and myelin.

SPHINGOSINE

$CH_3-(CH_2)_{12}-CH=CH-CH(OH)-CH-NH-C(=O)-R$

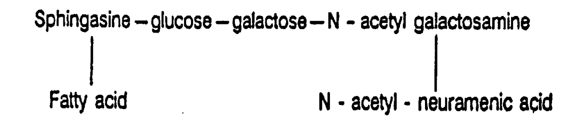
FATTY ACID

CH_2OH, HO, H, OSO_4, H, H, H, OH, $-O-CH_2$

Structure of a sulfatide (cerebroside sulfate)

Gangliosides:

- They are more complex glycolipids.
- Gangliosides contain N-acetyl galactosamine (Gal NAC) and N-acetylneuraminic acid (NANA), so they are acidic.
- Gangliosides are particularly prevalent in the gray matter of the brain and nerve synapses.
- Small amount of ganglosides is present in extraneural tissues.
- Because they are present in the nerve endings they are important in the transmission of nerve impulses.



Ganglioside

Lipoproteins:

Lipoproteins are macromolecular complexes that serve in the plasma as the transport vehicle of insoluble lipids.

For descriptive purposes they have been classified into five classes on their density (g/ml), flotation characteristics, and mobility on paper or agar gel electrophoresis.

Distrbution of lipoproteins in the body:

1. In tissue cells:
   - a) Cell membrane. b) Nucleus.
   - c) Mitochondria d) Microsomes.
2. In organs:
   - a) Lung tissue as thrompoblastic protein.
   - b) Eye (layer of rods in retina)
3. In blood: Most of the blood lipids are carried as lipoprotein complexes.

The function of lipoproteins are not only restricted to the transport of water insoluble lipids but also play an important role in cholesterol and triacylglycerol metabolism.

Lipoprotein *can be classified by flotation rate in NaCl solution using ultracentrifugation to:*

1- Chylomicrons:

- In these lipoproteins the arrangement of lipids and proteins takes place in two phases: the "core" or center of the particle is occupied by neutral lipids (triacylglycerol, cholesterol) and the "surface" or water-lipid interface is occupied by apoproteins, phospholipids, and unesterified cholesterol.

Triacylglycerol constitute	**85 to 95**	**%**
Phospholipids	**5 to 10**	**%**
Cholesterol	**3 to 5**	**%**

- Their apoprotein content is 1 to 2 % of particle mass and consists predominantly of apo B, some apo A and the various C apoproteins.
- Chylomicrons are acted upon by a number of lipoprotein Lipases that hydrolyze the triacylglycerol component to monoacylglycerol, glycerol and free fatty acids, which can then be taken up at the cellular level for energy metabolism or for resynthesis of triacylglycerol for storage.

2- <u>Very low density lipoprotein (VLDL)</u>:

- There is a wide range of VLDL particle sizes, which are synthesized and released from the liver.
- VLDL transport hepatic synthesized triacylglycerol and cholesterol, which are therefore, called endogenous lipids.
- The major lipid fraction contains 60-70% triacylglycerol.
- Cholesterol and Phospholipids are minor constituents.
- A member of important apolipoproteins are contained in VLDL (10%) the most notable being apo B-100 apo C and apo E. As with chylomicrons.
- The bigger VLDL particles contain relatively less apoprotein, phospholipids and cholesterol (surface material) and more triacylglycerol (core constituent) than the smaller particles.
- The "increased" apoprotein content of smaller particles is largely relative owing to the loss of triacylglycerol.

3- <u>Intermediate density lipoprotein (IDL)</u>:

- This fraction is not usually considered a separate species of lipoprotein but rather occurs during the transition of VLDL to LDL.
- IDL is not normally identified in plasma because of its rapid turn over of 2 to 6 hours.

- Each particle of IDL contains approximately :--
  40% triacylglycerol.
  30% Cholesterol,
  20% phospholipids.
- The ratio of esterified to free cholesterol is lower than in VLDL the sphingomyclin : lecithin ratio is about three times that found in the VLDL particle.
- IDL is further catabolized by lipoprotein lipase to form the cholesterol rich LDL particle.

4- Low density lipoprotein (LDL):

- LDL constitutes about 50% of the total lipoprotein mass in human plasma.
- The approximate lipid composition is
  45% cholesterol ***(esterified: non-esterified molar ratio, 2 : 3)***
  20 to 30% phospholipids (***lecithin:sphengomyclin ratio,2:5)***,
  5 to 10% triacylglycerol.
- More than 95% of the protein of LDL is accounted for by apo B, where as only very small amounts of apo C are found in the LDL density range.
- LDL are normally formed by hepatic delipidation of IDL which is controlled by hepatic lipoprotein lipase intravascularly.
- The bound LDL is internalized and subjected to lysosomal degradation that ultimately hydrolyses the apo B to amino acids, and the esterified cholesterol is hydrolyzed to free cholesterol, which enters the cytoplasm.
- The release of free cholesterol is responsible for three regulatory responses in cholesterol metabolism:
  a) Suppression of 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (HMG-CoA) rate controlling enzyme in the cholesterol biosynthetic pathway, thus new cholesterol synthesis is available.

b) Activation of LCAT activity to esterify excess cholesterol for intracellular storage as cholesterol ester droplets.

c) Decrease the number of LDL receptors on the plasma membrane to prevent then over accumulation of intracellular cholesterol through the receptors pathway.

5- High density lipoprotein (HDL):

- HDL consists of a number of heterogeneous particles that vary in respect to size and content.
- Both the liver and the intestine are involved in the production of HDL.
- HDL components may also arise from the catabolism of VLDL and chylomicrons.
- A small amount of (apo C and apo E),
  20% cholesterol (mostly esterified),
  30% phospholipids
  only traces of triacylglycerol.
- HDL is often separated into two subclasses: HDL2 (density 1.063 to 1.125 g/ml) and HDL3 (density 1.125 to 1.210 g/ml) which differ in composition and particle size.
- HDL appears to be important in cholesterol efflux from tissues thereby reducing the amount of cholesterol stored there.
- HDL play a major role as a scavenger of lipid and apolipoprotein during the normal catabolism of chylomicrons and VLDL. HDL is also known to play an important part as a plasma reservoir for apo CII.
- Little is known about the sites of HDL catabolism however the liver and kidneys are probably the sites.

Lipoprotein (A):

- It exhibits many of the properties of LDL, but it should be considered as an additional lipoprotein class.

- LP (a) can be isolated from plasma between d 1.05 and 1.12 and exhibits pre-B mobility on agarose electrophoresis.
- Also the lipid compositions of LDL and LP (a) are similar but their protein components are quiet different
- LP (a) when dilapidated contains 65% apo B 15% albumin and unique apo-protein called apo LP (a).
- LP (a) has been shown in 75-78% of two randomly selected populations. Its concentration does not correlate with age sex, lipid concentration or exercise.
- Recently, LP (a) is considered as an independent risk factor for coronary heart disease and in particular for myocardial infarction.

Lipoprotein (X):

- It is an abnormal lipoprotein containing a high proportion of phospholipids and unestrified cholesterol.
- LP (x) lack apo A and have suggested that the abnormal lipoprotein is the result of synthesis of an altered apo A that does not bind lipid.
- LP (x) found in patients with obstructive jaundice. It contains about 65 percent lecithin, 25 to 30 percent unesterified cholesterol, 2 percent cholesterol ester, and 6 percent protein.
- About 80 percent of protein in LP (x) is apo C, and the rest is albumin.
- Apo C, is believed to be located on the surface of the LP (x) particle, since it can be identified immunochemically on the intact particle.
- The albumin, on the other hand, is presumably located in the interior of the particle; it can be identified in LP (x) only after delipidation or attack by phospholipases.

Apolipoproteins

- Apolipoprotein are several specific proteins which form with lipids complexes known as lipoprotein.
- The apoplipoproteins play important roles in lipid transport by activating or inhibiting enzymes in lipid metabolism and/or by binding lipoproteins to cell surface.
- An alphabetical classification proposed identifies five major groups of apolipoproteins and a number of subgroups according to their structures, physiochemical behaviour, and their function.

1- Apolipoprotein A (APO A):

- Three apoproteins, designed apo A-I, which activates lecithin cholesterol acyl transferase (LCAT) enzyme.
- Apo A-II which inhibits LCAT enzyme and is useful in lipid transport.
- Apo A-III, can be isolated from human HDL.
- They are easily separated by various chromatographic techniques.
- They differ in amino terminals, amino acid composition, and immunologic properties.
- They are all soluble in water and urea solutions and contain glutamine at their carboxyterminals.

2- Apolipoprotein B (APO B):

- Apo B is the major protein constituent of LDL, VLDL and chylomicrons averaging about 90%, 40% and 20% of their total masses, respectively.
- Its concentration in human plasma ranges between 700 and 1000 mg/l.

- Carbohydrates (mannose, fucose, glucosamine, glucose, galactose and sialic acid) constitute about 5 percent of the mass of apo B.
- In the rat apo B is synthesized both in the liver and in the intestine and enters the circulation in either chylomicrons or VLDL.
- There are two major components of apo B:
  a) Apo B-100: which is synthesized in the liver and helps in lipid transport and clearance.
  b) Apo B-48: which is synthesized in the intestine and acts as chylomicron transport.

3- Apolipoprotein C (APO C):

- At least three different apoproteins belong to this group:
  a) C-I acts as LCAT activator.
  b) C-II acts as lipoprotein lipase activator.
  c) C-III acts as lipoprotein lipase inhibitor.
- Their masses range between 8 and 10×10^3 Dalton
- These apoproteins are best separated by ion-exchange chromatography by use of DEAE cellulose.
- In fasting human plasma the C apoproteins are found mainly in VLDL and HDL (predominantly HDL2).
- They play an important role in the metabolism of triacylglycerol-rich lipoproteins.
- Apo C-II is a specific co-factor essential for triacylglycerol hydrolysis by extrahepatic lipoprotein-lipase.
- Apo C-III may serve as a specific inhibitor of the lipoprotein-lipase system.
- In rats apo C is synthesized and secreted by the liver, but not the intestine.

4- Apolipoprotein D (APO D):

- This apoprotein is isolated as a minor constituent from human HDL, especially HDL_3.

- It participates in the activation of the LCAT system and may be a specific carrier of the lysolecithin formed after LCAT has acted on HDL.

5- Apolipoprotein E (APO E):

- Also found in human plasma is an arginine-rich apoprotein (apo E), with a molecular weight of 33.000
- it has amino-terminal of lysine and a carboxy-terminal sequence of leucine-serine-alanine.
- It constitutes about 5 to 10 percent of normal VLDL and is found in excess amounts in-patients with Type III hyperlipoproteinemia.
- It also presents in minor quantities, in LDL and HDL.

Lipoprotein lipases:

- In fasting human plasma, lipolytic activity is barely detectable.
- A few minutes following the intravenous injection of heparin, several lipolytic activities are discerned.
- At least two triacylglycerol hydrolases are detected in this so-called post-heparin plasma.
- They differ in their pH optimum, inhibition by protamine or concentrated saline solution and activation by specific apoprotein co-factors.

1- Extrahepatic lipoprotein lipase:

- This enzyme, derived mainly from adipose tissue, is operative in the hydrolysis of chylomicron and VLDL triacylglycerol.
- It is normally located on the surface of endothelial cells of adipose tissue and of skeletal and heart muscles.
- Hydrolysis of chylomicron triacylglycerol occurs following the attachment of these particles to the capillary endothelial cells.

- Phospholipids and apo C-II are essential co-factors for triacylglycerol hydrolysis by this enzyme.
- In familial Type I hyperlipoproteinemia there is a complete absence of extrahepatic lipoprotein-lipase activity.

2- Hepatic lipoprotein lipase:

- This enzyme is predominantly associated with the hepatocyte outer membrane.
- It has only a limited capacity to hydrolyze significant amounts of triacylglycerol from intact glyceride-laden lipoproteins.
- It has been postulated that this enzyme is operative in the conversion of IDL into LDL particles.
- Since the activity of hepatic lipoprotein-lipase is independent of the presence of apo C, it can proceed in the apo C-poor IDL.

3- Post heparin phosopholipase:

- Another lipolytic activity present in post-heparin plasma is a phospholipase which hydrolyzes fatty acids in the 2-position of phosphatidylcholine and phosphatidyl-ethanolamine.
- The enzymatic activity results in formation of lysophosphatide compounds, mainly lysolecithin.
- In the rat most of the phospholipase activity originates in the liver.
- Recent studies have indicated that hydrolysis of lecithin to lysolecithin may account for the disappearance of some phospholipids from VLDL after interaction of this lipoprotein with post-heparin plasma.

4- Lecithin cholesterol acyl transferase (LCAT):

- The enzyme LCAT is synthesized and secreted by the liver.
- Normally present in human plasma, this enzyme system catalyzes the esterification of cholesterol by promoting transfer of fatty acids from lecithin to cholesterol, which results in the formation of lysolecithin and cholesterol ester.

- The enzyme is synthesized in the liver and circulates in plasma with HDL, which seems to be the preferred substrate.
- It is activated by apo A-I. Recently, it was suggested that this enzyme system also play a role in removing surface material of Chylomicrons and VLDL.
- LCAT may also be involved in removal of excess free cholesterol and lecithin from the circulation.

Hyperlipidemia and hyperlipoproteinemia:

Hyperlipidemia is defined as an elevation of plasma lipids. These lipids include cholesterol and its esters. Triacylglycerol and phospholipids.

Hyperlipoprteinemia are conditions in which the concentration of cholesterol or Triacylglycerol carrying lipoproteins in plasma exceeds an arbitrary normal limit.

Hyperlipemia is restricted to those conditions in which levels of Triacylglycerol in plasma are increased.

Many pathogenic mechanisms may be responsible for the resulting hyperlipoproteinemia.

These include:-

i) Defects that lead to increased influx of exogenous cholesterol or of triacylglycerol from the intestine as chylomicrons.
ii) Increased release of VLDL containing cholesterol or triacylglycerol from the liver and small intestine.
iii) Defects in enzymes that catabolize lipoproteins, and abnormalities of the solubility of lipoproteins or in their structures.
iv) Other modifying factors include:
    1. The general state of carbohydrate or protein metabolism.
    2. The activity of hormones that promote lipogensis or lipolysis in muscle, liver and adipose tissue.

The simplest *nomenclature* for defining the type of lipoproteins present in excess is the phenotyping system. This *classification* is based on the difference in migration of the different lipoproteins in lipid electrophoresis:

Phenotype [I] Excess Chylomicrons.
Phenotype [II a] Excess LDL.
Phenotype [II b] Excess LDL and VLDL.
Phenotype [III] Excess cholesterol-rich VLDL remnants.
Phenotype [IV] Excess VLDL.
Phenotype [V] Excess chylomicrons and VLDL.

Although widely used, this system offers no information about the origin of different types of hyperlipoproteinemia. Three categories of causation however have been identified; genetic, secondary and dietary.

DERIVED LIPIDS

– They are produced by hydrolysis of simple and compound lipids. They may be:

– *Fatty acids, Glycerol, Alcohol's, Steroids, Lipid soluble vitamins, Bile salts, Soponins and cardiac glycosides., and Carotenoids.*

– They are derived from simple and compounds lipids (1, 2, and 3) or associated with lipids (4, 5, and 6).

Steroids, carotenoides, fat-soluble vitamins and high molecular weight alcohols constitute "The unsaponifiable fraction of fats (USF)", since they can not be hydrolyzed by alkali, so they can be separated from other types of fat by saponification.

Steroids

– Steroids have a similar cyclic nucleus which is known as steroid nucleus: (cycloperthydrocyclopentanophenantherene ring) = sterone

Cyclopentanoperhydrophenanthrene nucleus

- The steroid nucleus is composed of 4 rings, A, B, C and D.
- Ring A, B and C contains six carbon atoms, whereas D ring contains five.
- An angular methyl group C_{19} is attached at the junction of the A and B rings, and a second angular methyl group C_{18} is attached at the junction of the C and D rings.

Steroids included:

i) Sterols.
ii) Bile acids and salts.
iii) Steroid hormones.
iv) Vitamin D.
v) Saponines and cardiac glycosides.

Sterols:

They are a class of steroids that contain a hydroxyl group at C_3 *i.e.* it is an alcohol and aliphatic chain of at least eight carbon atoms at C_{17}.

21 22 20 23 26 25 17 24 27

Types of sterols:

1- Animal sterols:

Cholesterol and its derivatives:

i) Dihydrocholesterol.
ii) Coprosterol.
iii) 7-dehydrocholestrol.

2- Plant sterol:

i) Ergosterol.
ii) 22-dihydroergosterol.

Cholesterol:

It is the main sterol in human tissue. The hydroxyl group attached to C_3 is β-oriented and there is a double bond between C_5 and C_6 at ring B.

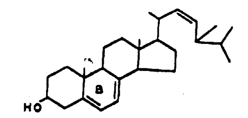
HO

Cholesterol

- Foods that are derived from animal products contain cholesterol, particularly eggs, dairy products such as butter, cheese and cream, and most meat.
- Cholesterol may be found free or esterified *i.e.* there is a fatty acid attached to the OH group, e.g. cholesteryl oleate and cholesteryl linoleate.
- Cholesterol is found in membrane lipids, also it forms with its esters an appreciable amount in the plasma lipoproteins.
    - It is present in all cells of the body especially:
        - Nervous tissues. - Brain.
        - Suprarenal gland. - Bile.
    - It is present in blood *(upto 200 mg/dl).*
    - It is formed in the body from acetyl Co-A.
    - High level of cholesterol in blood lead to its precipitation in the wall of blood vessels in a disease called *(Atherosclerosis).*
    - High levels of blood cholesterol also lead to stones in gall bladder *(gall stones).*
- Cholesterol is the precursor of other sterols in the body e.g. steroid hormones and vitamin D.

Plant sterols:

- They contain phytosterols instead of cholesterol
- The most abundant of phytosterol is ergoesterol.
- Present in lower plants as yeast and moulds.
- Ergosterol is a provitamin D_2.
- 22-dihydroergosterol is a provitamin to D_4.



Ergosterol

Steroid hormones:

Cholesterol can be converted into steroid hormones. The steroid hormones are: -

* ***Sex hormones***: that includes male and female hormones.
* ***Adrenocortical hormones***: those include mineralocorticoids and glucocorticoids.

I. Male sex hormones: *(19-carbon atoms)*

1. Testosterone:

It is synthesized in the interstitial tissue of the testis by the Lydig cells.

Testosterone

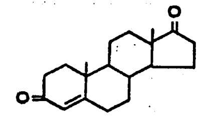
It is composed of is carbon atoms, there is a hydroxyl *group* attached to C_{17}.

2- Androstenediole:

It is synthesized in the testicular and suprarenal cortex.

Androstenediole

3- Androstenedione:



Androstenedione

Male sex hormones (androgens) are involved in:

1. Sexual differentiation.
2. Spermatogenesis.
3. Development of secondary sexual organs and male musculature..
4. Male - pattern behavior.
5. Protein anabolic hormone (nitrogen retention), they cause retention of Na, K, Cl and P.

II. Female sex hormones:

1. Estrogens:

OH
HO
Estradiol

O
HO
Estrone

OH
OH
HO
Estriol

- Estrogens contain 18 carbon atoms.
- The ring A is unsaturated, it is a benzene ring.
- The ovaries synthesize them.
- Their main function is to prepare the structural determinants of the female reproductive system.

  Functions :

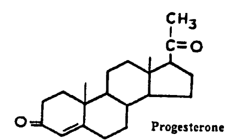
  1. Development of female secondary characters.
  2. Maturation of the germ cells.
  3. Suppression of production of FSH.
  4. Proliferation and vascularization of uterus.

2-Progesterone:

- Progesterone is formed of 21 carbons.
- It is produced and secreted by the corpus luteum and placenta.

Functions :

1. Develop the uterine endometrium to allow for implantation of the fertilized ovum, and maintain pregnancy.
2. Provide the hormonal timing for ovulation.
3. It is considered as a precursor of many important steroids as other sex hormones and cortical hormones.
4. Stimulate mamary glands *(acinar protein).*
5. Essential for continuation of both ovulation and leutinizing hormone production.



III. Adrenocortical hormones: *(corticoids)*

The cortex of the suprarenal glands (adrenal gland) secretes them.

They are of 2 types:

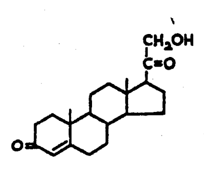
1. Mineralocorticoids.
2. Glucocorticoids.
3. Corticoids act as sex hormones *(Androstenediol, Androstenedione).*

1. Mineralocorticoids *"N_{21} carbons":*

- Hormones effect electrolytes and water metabolism.
- They are synthesized and secreted by zona glomerulousa cells of the adrenal cortex.
- There are 2 mineralocorticoides: Aldosteron and 11-Deoxy-corticosterone.

The functions of mineralocorticoids include:

1. They act in the kidney to stimulate Na^+ transport by the renal tubules causing Na^+ retention.
2. They promote K^+, H^+ and NH_4^+ excretion by kidney. In these actions, aldosterone is more potent than 11- deoxy corticosterone.



11-deoxy-corticosterone

Aldosterone (Aldehyde form)

Aldosterone (Hemiacetal form)

2. **Glucocorticoids** *"21 carbons":*

- Hormones effect carbohydrates, lipid and protein metabolism.
- The cells of zona fasciculata of the adrenal cortex produce them.
- Cortisone is the predominant glucocorticoid in humans, and corticosterone is less abundant in human.
- Corticosterone is less abundant in human.

<u>*Functions :*</u>

1. Stimulation of gluconeogenesis *(synthesis of glucose from non-carbohydrate sources).*
2. They lower the glucose uptake by the tissues and decrease glucose oxidation.

The result of 1. and 2. is rising of blood glucose level.

3. They inhibit lipogenesis *(lipid synthesis)*. and cause redistribution of fat in the body i.e., they mobilize fat from extremities and deposit it in the trunk and face. The result is thin upper and lower limbs with obesity in the trunk and moon face.
4. They have a mineralocorticoid action i.e. they retain Na^+ and excrete K^+, H^+ and NH_4^+

Cortisol (17-hydroxycorticosterone)

Cortisone (11-dehydro-17-hydroxycorticosterone)

Lipid Soluble Vitamins (from steroids)

1. Vitamin D:

Sources:-

From the plant sterol Ergosterol and 22-dihydro-Ergosterol by the action of ultraviolet rays (from sunlight) producing vitamin D_2 and D_4.

Ultraviolet rays
" sunlight "

Ergosterol — **Vitamin D_2**

(provitamin D_2) — *(Claciferol)*

From the cholesterol derivative 7-dehydrocholesterol (that is present in the subcutaneous tissue). By the action of ultraviolet rays (from sunlight), it gives vitamin D_3.

Ultraviolet rays
" sunlight"

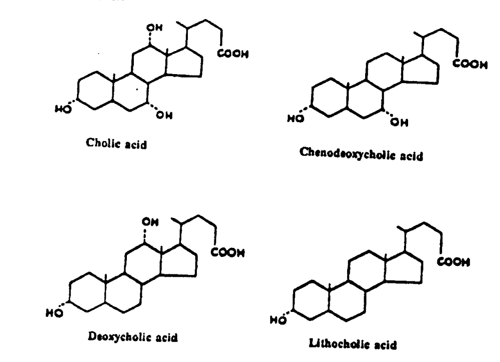
7-dehydrocholesterol — **Vitamin D_3**

(provitamin D_3) — **(Cholecalciferol)**

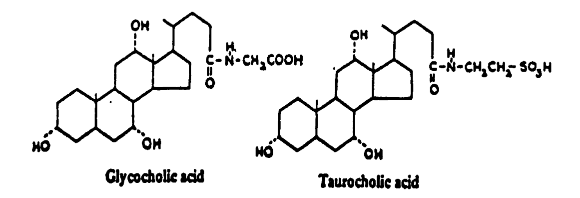
Vitamin D is important for bone and teeth mineralization. and in calcium and phosphorus metabolism.

Bile Acids

- They are the main metabolic products of cholesterol.
- The bile acids in human are cholic, chenodeoxycholic, deoxycholic and lithocholic acids that are derivatives of cholanic acid.



- Bile acids contain twenty-four carbon atoms.
- The four bile acids differ only in the number of hydroxyl groups that are attached to the steroid nucleus.
- Both glycine and taurine can condense with bile acids in the liver to form glyco- or tauro-conjugate bile acids.
- Both glycine and taurine are joined in amide linkage with the carboxyl group *(C_{24})* forming bile salts.



The functions of bile salts are:

1. Activation of pancreatic lipase.
2. They lower the surface tension of lipids causing their emulsification and thus increase the surface area for lipid digestion by lipase.
3. They have a hydrotropic property *i.e.,* they make water insoluble substance more soluble in aqueous medium and this facilitates their absorption of fat-soluble vitamins.
4. They keep cholesterol in solution. If they are low in concentration, cholesterol will deposit in the form of stones.
5. They have a choleritic action *i.e.,* they stimulate bile secretion from the liver.

N.B. The gall bladder wall absorbs water from the bile contained in it, thus producing a highly concentrated bile. It also absorbs certain inorganic salts. Mucin is added to the bile in the gall bladder.

Composition of bile

Human bile is clear viscous fluid, golden yellow. or brownish yellow in colour.

Composition of hepatic bile as compared to bladder bile is shown in the following table

Constituent	***Hepatic bile "% of total bile"***	***Bladder bile % of total bile"***
- Water	97.00	85.92
- Solids	2.52	14.08
-Bile acids	1.93	9.14
- Mucin and bile pigments	0.53	2.98
- Cholesterol	0.06	0.26
- Esterified and non-esterified FA	0.14	0.32
- Inorganic salts	0.84	0.65
- specific gravity	1.01	1.04
- pH	7.1-7.3	6.9-7.7

Bile acids:

- The parent bile acids found in human bile are hydroxylated cholanic acids.
- Qualitatively, the principal acids are cholic acids chenodeoxycholic acid and deoxycholic acid.

Bile acids can be classified into primary and secondary bile acids.

Primary bile acids:

- They are synthesized in the liver from cholesterol by several intermediate steps.
- *Cholic acid* is the primary one found in the largest amount in the bile itself.
- The other primary acid is *chenodeoxycholic acid*.
- These two acids are formed from a common precursor, which is derived from cholesterol.
- In human and other mammalian bile, the acids are conjugated with glycine and taurine in variable ratios.
- These conjugations are present as sodium and potassium salts hence the term "bile salts"

Secondary bile acids:

- A portion of the primary bile acids in the intestine may be subjected to some further changes by the activity of the intestinal bacteria.
- These changes include deconjugation and 7 α-dehyroxylation which produce *deoxycholic acid* from cholic acid and *lithocholic acid* from chenodeoxycholic acid.

Circulation of bile salts:

During normal digestion, when the bulk of the bile is delivered to the intestine, about 99% of the acids are reabsorbed by

the portal blood and are removed by the liver and re-excreted in the bile. This is known as enterohepatic circulation.

This circulation of the bile salts is so efficient that each day the relatively small amounts of bile acids *(about 3-5 g)* can be cycled through the intestine *6-10* times with only a small amount lost in feces *i.e.,* approximately 1% per cycle through the enterohepatic circulation. This small amount represents a major pathway for the elimination of cholesterol.

However, each day, an amount of bile acids equivalent to that lost in feces is synthesized from cholesterol by the liver, so that a pool of bile acids of constant size is maintained.

Functions of bile

1. Emulsification:

- Bile salts have a considerable ability to lower the surface tension.
- This property accounts for the emulsification of fat with concurrent production of a great surface area, which enables lipase and other enzymes to act more efficiently.
- The presence of bile in the intestine Is important to accomplish the digestion and absorption of fats as well as fat soluble vitamins (aggregation of bile salts into micelles and the formation of mixed micelles with the products of fat digestion are important in facilitating absorption of lipids).
- When the fat digestion is impaired other food stuffs are also poorly digested, since the fat covers the food particles and prevents enzymes from attaching them.
- Under these conditions the activity of the intestinal bacteria causes considerable putrefaction and production of gas.

2. **Neutralization of acid chym:**

The bile is a reservoir of alkali, which helps to neutralize the acid chym from the stomach.

3. **Excretion:**

Bile is an important vehicle of cholesterol excretion and also for excretion of many drugs, toxins, bile pigments, and various inorganic substances such as copper, zinc and mercury.

4. **Cholesterol solubility:**

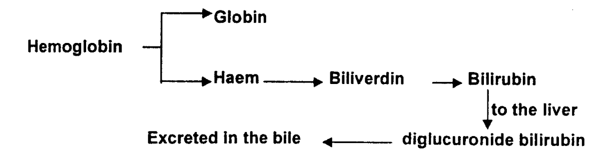
- The large quantities of cholesterol present in bile of humans are solubilized in the water-soluble mixed micelles (formed of cholesterol, bile salts and lecithin).
- However, the actual solubility of cholesterol in the bile depends on the relative proportions of bile salts, lecithin, and cholesterol.
- Also the solubility depends on the water content of bile.

5. **Bile salts**

They have cholagogue effect *i.e.* stimulate the further production of bile. They also stimulate peristalsis.

6. **Excretion of bile pigments:**

Bile pigments *(bilirubin and biliverdin)* are originated from catabolism of hemoglobin and conjugated by the liver then excreted in the bile



Excessive amounts of bile pigments in bile and infection cause the precipitation of bile pigments in the form of gall stones *(pigment stones).* While increased amounts of bile pigments in blood is called jaundice.

Types of jaundice:

* ***Hemolytic jaundice*** *(prehepatic):* due to excessive blood haemolysis. The bile pigments increased in blood and is in unconjugated form.
* ***Hepatic jaundice:*** due to liver disease (*e.g.* *infective hepatitis).* It is characterized by increase of both conjugated and unconjugated bile pigments in blood.
* ***Obstructive Jaundice*** *(post-hepatic Jaundice):* due to obstruction of common bile duct (*e.g.* by a stone or cancer head pancreas). Bile pigments increased in blood and are conjugated to glucuronate.

Gallstones:

- Gallstones occur not infrequently in the gall bladder and sometimes in the bile ducts.
- There may be one stone or great number of small stones.
- It is believed that at sometime during life of patient with gallstone there is formed an abnormal bile that has become supersaturated with cholesterol.
- With time, various factors such as infection for example., cause precipitation of cholesterol as crystals.
- Unless the newly formed crystals are excreted into the intestine with bile, the crystal will grow to form stone.

PROTEINS

PROTEINS

Proteins are the most abundant organic molecules in cells constituting 50 or more of their dry weight, they are found in every part of every cell, since they are fundamental in all aspects of cell structure and function.

There are many different kinds of proteins, each specialized for a different biological function. Moreover, most of the genetic information is expressed by proteins.

Functions of proteins:

Proteins play crucial roles in virtually all biological processes. The significance and remarkable scope of their functions are exmplified in:-

1) Enzymatic catalysis:

Nearly all chemical reactions in biological systems are catalyzed by specific macromolecules called enzymes that are protein in nature.

2) Transport and storage:

Many small molecules and ions are transported by specific proteins for example, haemoglobin transports oxygen in erythrocytes, whereas myloglobin transports oxygen in muscle. Iron is carried in plasma of blood by transferrin and is stored in the liver as a complex with ferritin, a different protein.

Other important proteins act to transport horomones in blood from their sites of synthesis to their sites of action. Many drugs and toxic compounds are transported bound to proteins

3) Coordinated motion:

Proteins are the major component of muscle. Muscle contraction is accomplished by the sliding motion of two kinds of protein filaments that are myosin and troponin.

4) **Mechanical support:**

The strength of skin and bone is due to the presence of collagen, an elongated protein that forms fibers.

5) **Immune protection:**

Mtibodies are highly specific proteins that recognize and combine with such foreign substances as viruses, bacteria and cells from other organisms.

6) **Generation and transmission of nerve impulse:**

The response of nerve cells to specific stimulus is mediated by receptor proteins. For example rhodopsin is the photoreceptor protein in retinal rod cells

7) **Control of growth and differentiation:**

– Controlled sequential expression of genetic information is essential for the orderly growth and differentiation of cells. Only a small fraction of the genome of a cell is expressed at any one time.

– Hundreds of different proteins have been isolated in pure crystalline form. The molecular weights of proteins are very high, but on acid hydrolysis they all yield a group of simple organic compounds of low molecular weight the amino acids.

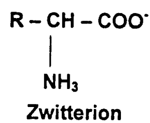
AMINO ACIDS

- Twenty different α-amino acids are commonly found as the building blocks of proteins.
- Besides the 20 amino acids found as building units of proteins, many additional biologically occurring amino acids serve other functions in cells.
- An amino acid consists of an amino group, a carbonyl group, a hydrogen atom, and a distinctive a group bonded to a carbon atom, which is called the a carbon.
- An R group is referred to as a side-chain.

$$\begin{array}{c} NH_2 \\ | \\ R - C - COOH \\ | \\ H \end{array}$$

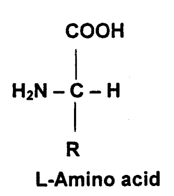
α-Amino acid

- Amino acids in solution at neutral pH are predominantly dipolar ions (zwitterions) rather than un-ionized molecules.
- In the dipolar form of an amino acid, the amino group is protonated (NH_3^+) and the carboxyl group is dissociated (COO^-).
- So, when a crystalline zwitterionic amino acid is dissolved in water, it can act either as an acid (proton donor) or as a base (proton acceptor).
- Substnces having this property are amphoteric (Greek amphi = both) and are called ampholytes.



Zwitterion

- The tetrahedral array of four different groups about the α-carbon atom confers optical activity on amino acids.
- The two mirror-image forms are called the L-isomer and the D-isomer.
- Only the L-amino acids are constituents of proteins.



L-Amino acid

- Twenty kinds of side chains varying in size, shape, charge, hydrogen-bonding capacity and chemical reactivity are commonly found in proteins.
- Indeed, all proteins in all species from bacteria to man, are constructed from the same set of twenty amino acids
- The remarkable range of functions mediated by proteins result from the diversity and versatility of these twenty kinds of building blocks.

Classification of amino acids:

The amino acids present in proteins may be divided into 2 broad groups on the basis of whether R groups attached to the α-carbon atoms are polar or non polar.

From the biological point of view, there are two groups, essential and non-essential amino acids.

- The essential amino acids are ten which can not be formed by the animal body and must be taken in the diet.
- These are threonine, methionine, valine, leucine, isoleucin, lysine, arginine, histidine, phenylalanine and tryptophan.
- The biological importance of a protein depends on its content of the essential amino acids.

L-α-Amino acids present in proteins

Name	Symbol	Structural formula
With Aliphatic Side Chains		
Glycine	Gly [G]	$H-CH(NH_2)-COOH$
Alanine	Ala [A]	$CH_3-CH(NH_2)-COOH$
Valine	Val [V]	$(H_3C)_2CH-CH(NH_2)-COOH$
Leucine	Leu [L]	$(H_3C)_2CH-CH_2-CH(NH_2)-COOH$
Isoleucine	Ile [I]	$H_3C-CH_2-CH(CH_3)-CH(NH_2)-COOH$

Name	Symbol	Structural formula
With Side Chains containing Hydroxylic (OH) Groups		
Serine	Ser [S]	CH_2-CH-COOH OH NH_2
Threonine	Thr [T]	CH_3-CH-CH-COOH OH NH_2
With Side Chains Containing Sulfur Atoms		
Cysteine	Cys [C]	CH_2-CH-COOH SH NH_2
Methionine	Met [M]	CH_2- CH_2-CH-COOH S-CH_3 NH_2
With Side Chains Containing Acidic Groups or Their Amides		
Aspartic acid	Asp [D]	HOOC-CH_2-CH-COOH NH_2
Asparagine	Asn [N]	H_2N-C-CH_2-CH-COOH O NH_2
Glutamic acid	Glu [E]	HOOC-CH_2-CH_2-CH-COOH NH_2
Glutamine	Gln [Q]	H_2N-C-CH_2-CH_2-CH-COOH O NH_2
With Side Chains Containing Basic Groups		
Arginie	Arg [R]	H-N-CH_2-CH_2-CH_2-CH-COOH C=NH NH_2 NH_2

Name	Symbol	Structural formula
Lysine	Lys [K]	CH_2-CH_2-CH_2-CH_2-CH-COOH (with NH_2 on the first CH_2 and NH_2 on CH)
Histidine	His [H]	Imidazole ring (HN, N)–CH_2-CH-COOH (with NH_2 on CH)
With Aromatic Rings		
Phenylalanine	Phe [F]	Benzene ring–CH_2–CH–COOH (with NH_2 on CH)
Tyrosine	Tyr[Y]	HO–benzene ring–CH_2–CH–COOH (with NH_2 on CH)
Tryptophan	Trp[W]	Indole ring (N–H)–CH_2–CH–COOH (with NH_2 on CH)
Amino acids		
Proline	Pro [P]	Pyrrolidine ring (N–H)–COOH

Non protein amino acids:

- In addition to the 20 common amino acids of proteins over 150 other amino acids are known to occur biologically in free or combined form but never in proteins.
- Some non-protein amino acids are important precursors or intermediates in metabolism. Thus, β-alanine is a building block of the vitamin pantothenic acid, homocysteine and homoserine are intermediates in amino acid metabolism, citrulline and ornithine are inter mediated in the biosynthesis of urea.
- Other examples of non-protein amino acids include monoidotyrosine, diiodotyrosine, triodothyronine and thyroxine (precursors of thyroid hormones), and dopa (precursor of melanin and catecholamine).
- Also, y-amino butyric acid (formed from glutamic acid in brain tissue) serves as a chemical agent for the transmission of nerve impulses.

Selected examples of non-α-amino acids that perform important functions in mammalian metabolism

Common and Systematic Names	Formula	Significance
β-Alanine (3-aminopropanoic acid)	$CH_2(NH_2)$-CH_2-COOH	Part of coenzyme A and of the vitamin pantotheine
Taurine (2-aminoethylsulfonic acid)	$CH_2(NH_2)$-CH_2-SO_3H	Occurs in bile combined with bile acids
γ-Aminobutyric acid (4-aminobutanoic acid)	$CH_2(NH_2)$-CH_2-CH_2-COOH	Formed from glutamate in brain tissue
Homocysteine (2-amino-4-mercapto-butanoic acid)	$CH_2(SH)$-CH_2-$CH(NH_2)$-COOH	An intermediate in methionine biosynthesis
Homoserine (2-amino-4-hydroxy-butanoic acid)	$CH_2(OH)$-CH_2-$CH(NH_2)$-COOH	An intermediate in threonine, aspartate and methionine metabolism
Ornithine (2, 5-bisamino-pentanoic acid	$CH_2(NH_2)$-$(CH_2)_2$-$CH(NH_2)$-COOH	An intermediate in the biosynthesis of urea
Citrulline (2-amino-5-ureidopentanoic acid)	$CH_2(NH$-C(=O)-$NH_2)$-$(CH_2)_2$-$CH(NH_2)$-COOH	Intermediate in the biosynthesis of urea
Dopa (3, 4-dihydroxy-phenylalanine)	HO-(HO)C_6H_3-CH_2-$CH(NH_2)$-COOH	Precursor of melanin
3-Mono-iodotyrosine	HO-(I)C_6H_3-CH_2-$CH(NH_2)$-COOH	Precursor of thyroid hormones

Common and Systematic Names	Formula	Significance
3, 5-Diiodotyro-sine	HO-(3,5-diiodophenyl)-CH_2-CH(NH_2)-COOH	Precursor of thyroid hormones
3,5,3'-Triiodo-thyronine (T_3)	HO-(3-iodophenyl)-O-(3,5-diiodophenyl)-CH_2-CH(NH_2)-COOH	Precursor of thyroid hormones
Thyroxine (3,5,3',5'-tetra-iodothyronine) (T_4)	HO-(3,5-diiodophenyl)-O-(3,5-diiodophenyl)-CH_2-CH(NH_2)-COOH	Precursor of thyroid hormones

Biological classification of amino acids

Biologically amino acids may be classified into two classes:

A) Dispensable or non-essential amino acids:

These amino acids can be synthesized in the body and their absence from diet cannot cause growth failure e.g. glycine, alanine, serine, cysteine, cystine, nerleucine, aspartic glutamic, hydroxyglutamic acids, proline, hydroxyproline and tyrosine.

B) Indispensable or essential amino acids:

Are Those amino acids which cannot be synthesized in the body and their deficiency from diet causes growth failure e.g. pjenylalanine, tryptophan, threonine, methionine, valine, leucine, isoleucine and lysine.

Both groups of amino acids are required for the body but essentiality and non- essentiality is with respect to their presence or absence from diet.

Protein containing all essential amino acids in quite high proportions are considered proteins of high biological value

whereas others having them in low concentration or lacking some of them are proteins of low biological value.

Metabolic classification:

According to their metabolic fate, amino acids can be classified into 3 main groups:

1- Glucogenic amino acids:

These are amino acids which can give glucose inside the body e.g. glycine, glutamic acid, alanine and aspartic acid.

2- Ketogenic amino acids:

These are amino acid which can give ketone bodies inside the body e.g. leucine.

3- Glucogenic and ketogenic amino acids:

These are amino acids which can give glucose and ketone bodies inside the body e.g. phenylalanine, tyrosine and tryptophan.

Properties of amino acids

Physical properties:

1. Solubility:

Amino acids derived from proteins are α-amino acids. They are white crystalline substances, all soluble in water except cystine and tyrosine the latter being more soluble in hot than cold water. With the exception of proline they are all insoluble in alcohol and all are insoluble in ether but all soluble in solutions of strong acids and alkalies.

2. Taste:

Amino acids as glycine, alanine, serine and proline are sweet in taste but tyrptophan and leucine are tasteless whereas some like arginine are bitter.

3. Optical activity and spatial configuration:

With the exception of glycine, all naturally occurring amino acids are optically active due to asymmetry of the

α-carbon $R\text{-}\underset{}{\overset{NH_2}{\overset{|}{C}}}H\text{-}COOH$ and rotate the plane of polarized light either to the right or to the left i.e. dextrorotatory or levorotatory denoted ty (+) or (-) respectively.

On considering the spatial configuration of the amino acids, it is possible to have a D-form and a L-form of each.

$$\begin{array}{c} COOH \\ | \\ NH_2\text{-}C\text{-}H \\ | \\ CH_3 \end{array} \qquad\qquad \begin{array}{c} COOH \\ | \\ H\text{-}C\text{-}NH_2 \\ | \\ CH_3 \end{array}$$

L(+)-alanine **D(-)-alanine**

Most of the naturally occurring α-amino acids are the L-form. Certain D-amino acids have however been isolated from natural sources e.g. D-glutamic acid and D-valine.

4. Amphoterism, zwitterions and isoelectric point:

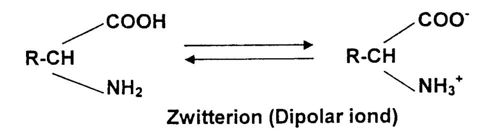
Since the amino acids contain both NH_2 and COOH groups they behave like amphoteric comounds i.e. ionizing both as acids reacting with alkalies and as bases reacting with acids e.g. with HCl it gives amino acids hydrochloride.

$$R\text{-}\underset{NH_2}{\underset{|}{C}}H\text{-}COOH + HCl \longrightarrow R\text{-}\underset{NH_3^+\ Cl^-}{\underset{|}{C}}H\text{-}COOH$$

Which ionizes giving a positively charged ion and a negatively Cl ion. But with alkalies as NaOH. The Na salt of the amino acid will be formed which ionizes giving a negative amino acid ion and a positive Na^+ ion.

$$R\text{-}CH(NH_2)\text{-}COOH + NaOH \longrightarrow R\text{-}CH(NH_2)\text{-}COO^- : Na^+$$

Therefore amino acids in acid medium ionize to positively charged ions and in alkaline medium to negatively charged ions. At a certain pH specific for each amino acid, the NH_2 and COOH groups are ionized to the same extent, the molecule is electrically neutral since it carries equal and opposite charges and in an electric field the ion will not migrate neither to the cathode nor to be anode. This pH is called the isoelectric point (I.E.P.) denoted by PI.



Zwitterion (Dipolar iond)

The amino acid ion possessing the above mentioned properties at the isoelectric point is known as the Zwitterion or Dipolar ion. When there are excess of NH_2 or COOH groups in the amino acid e.g. lysine and aspartic acid, it can be assumed that these are free and not Zwitterions.

Chemical reaction of amino acid:

II. Reactions of the amino group:

1) Amino acids react with acids to form salts:

$$R\text{-}CH(NH_2)\text{-}COOH + HCl \longrightarrow R\text{-}CH(NH_3^+\ Cl^-)\text{-}COOH$$

Amino acids amino acid medium are positively charged.

1) Amino acids react with acids to form salts:

$$R\text{-}CH(NH_2)\text{-}COOH + HCl \longrightarrow R\text{-}CH(NH_3^+\ Cl^-)\text{-}COOH$$

Amino acid in acid medium are positively charged.

2) They react with nitrous acid to give hydroxy acids:

$$R\text{-}\underset{\displaystyle NH_2}{\underset{|}{CH}}\text{-}COOH + HNO_2 \longrightarrow R\text{-}\underset{\displaystyle OH}{\underset{|}{CH}}\text{-}COOH + N_2 + H_2O$$

This reaction is not given by amino acids. Van Slyke collected the nitrogen gas and calculated the amino acid content of the solution.

3) Carbamino acid formation:

In alkaline solutions amino acids react with CO_2 to form carbamino acids.

$$R\text{-}\underset{\displaystyle NH_2}{\underset{|}{CH}}\text{-}COOH + CO_2 \longrightarrow R\text{-}\underset{\displaystyle NH\text{-}COOH}{\underset{|}{CH}}\text{-}COOH$$

This reaction is believed to play an important part in the transport of CO_2 by blood haemoglobin.

4) Acylation of amino acids:

Acetic anhydride or benzoyl chloride react with amino acids in presence of alkali to give the acyl derivative.

$$R\text{-}\underset{\displaystyle NH_2}{\underset{|}{CH}}\text{-}COOH + \underset{\text{Acetic anhydride}}{(CH_3\text{-}CO)_2O} \longrightarrow \underset{\text{Acetyl amino acid}}{R\text{-}\underset{\displaystyle NH\text{-}COCH_3}{\underset{|}{CH}}\text{-}COOH}$$

$$\underset{\text{Benzoyl chloride}}{C_6H_5\text{-}COCl} \longrightarrow \underset{\text{Benzoyl amino acid}}{R\text{-}\underset{\displaystyle NH\text{-}CO\text{-}C_6H_5}{\underset{|}{CH}}\text{-}COOH}$$

5) Hydrolysis of amino acids:

Certain amino acids undergo slow hydrolysis in the presence of bone black (C) and we get NH_3 and the corresponding hydroxy acid.

$$CH_3\text{-}\underset{NH_2}{CH}\text{-}COOH + HOH \longrightarrow CH_3\text{-}\underset{OH}{CH}\text{-}COOH + NH_3$$

Alanine **Lactic acid**

6) Reaction with acids:

e.g. glycine with benozic acid gives hippuric acid.

$$C_6H_5\text{-}COOH + H_2N\text{-}CH_2\text{-}COOH \xrightarrow{H_2O} C_6H_5\text{-}\overset{O}{\overset{\|}{C}}\text{-}\overset{H}{N}\text{-}CH_2\text{-}COOH$$

Benzoic acid **Glycine** **Hippuric acid**

7) Peptide linkage:

The amino group of an amino acid can combine with carboxyl group of a second amino acid. The product of combination is known as dipeptide and the linkage is a peptide linkage

$$H_2N\text{-}\underset{R_1}{CH}\text{-}COOH + H_2N\text{-}\underset{R_2}{CH}\text{-}COOH \xrightarrow{H_2O} H_2N\text{-}\underset{R_1}{CH}\text{-}CO\text{-}NH\text{-}\underset{R_2}{CH}\text{-}COOH$$

Dipeptide

8) Methylation of the amino group:

e.g. glycine gives betaine which can occur in zwiflerion form. Betaine can be transformed to choline by reduction.

$$NH_2\text{-}CH_2\text{-}COOH \rightarrow CH_3\text{-}\overset{CH_3}{\underset{CH_3}{N^+}}\text{-}CH_2\text{-}COOH \rightarrow CH_3\text{-}\overset{CH_3}{\underset{CH_3}{N^+}}\text{-}CH_2\text{-}CH_2OH$$

Glycine **Betaine** **Choline**

9) Oxidative deamination:

This is an important biologidal reaction of amino acids through which the amino acid is transformed to α-keto acid and the amino group is split as ammonia.

$$R\text{-}\underset{NH_2}{\overset{H}{C}}\text{-}COOH \xrightarrow{-H_2} \left[R\text{-}\underset{NH}{\overset{}{\underset{\|}{C}}}\text{-}COOH\right] \xrightarrow{+H_2O} R\text{-}\underset{O}{\underset{\|}{C}}\text{-}COOH + NH_3$$

α-Amino acid α-Imino acid α-Keto acid

III. Reactions of the acid group

1) They react with alkalies to form salts:

$$R\text{-}\underset{NH_2}{\underset{|}{C}H}\text{-}COOH + NaOH \longrightarrow R\text{-}\underset{NH_2}{\underset{|}{C}H}\text{-}COO^- Na^+$$

Amino acids ion is negatively charged in alkaline medium.

2) All amino acids react with alcohols to form esters in presence of HCl gas:

$$R\text{-}\underset{NH_2}{\underset{|}{C}H}\text{-}COOH + CH_3\text{-}CH_2OH \longrightarrow R\text{-}\underset{NH_2}{\underset{|}{C}H}\text{-}COOC_2H_5$$

Ethyl alcohol **Amino acid ester**

This property was made use of by Fischer who separated amino acids from each other by fractionally distilling their esters.

3) Formation of primary amines: by heating amino acids at high temperature in dry state or by heating with $Ba(OH)_2$ where the carboxyl group being disrupted to form CO_2 and a primary amine.

$$R\text{-}\underset{NH_2}{\underset{|}{C}H}\text{-}COOH \longrightarrow R\text{-}\underset{NH_2}{\underset{|}{C}H_2} + CO_2$$

4) Reactions of amino acids with formaldehyde:

The addition of an excess of neutral formaldehyde solution to a neutral amino acid solution, the first combines with the amino group and permits the COOH group to exert its maximum acidity and hence can be titrated with a standard base. This is the basis of Sorensen's method for estimating amino acids.

$$R\text{-}\underset{NH_2}{\underset{|}{C}}H\text{-}COOH + CH_2O \longrightarrow R\text{-}\underset{N{=}CH_2}{\underset{|}{C}}H\text{-}COOH + H_2O$$

5) Formation of esters with alcohols:

$$H_2N\text{-}\underset{R}{\underset{|}{C}}H\text{-}COOH + HO\text{-}C_2H_5 \longrightarrow H_2N\text{-}\underset{R}{\underset{|}{C}}H\text{-}CO\text{-}O\text{-}C_2H_3 + H_2O$$

Amino acid **Ethyl alcohol** **Ester**

6) Decarboxylation:

The amines formed are mostly of biological activity e.g. histamine

$$H_2N\text{-}\underset{R}{C}H\text{-}COOH \longrightarrow H_2N\text{-}CH_2\text{-}R + CO_2$$

Amino acid **Amine**

Imidazole ring $-CH_2-\underset{NH_2}{\underset{|}{C}}H-COOH$ $\xrightarrow[\text{decarboxylase}]{-CO_2}$ Imidazole ring $-CH_2-CH_2-NH_2$

Histidine Histamine

PEPTIDES

Definition:

In protein molecules, the α-carboxyl group of an amino acid is joined to the α-amino group of another acid by a peptide bond.

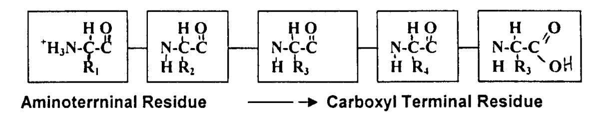
The formation of a dipeptide from two amino acids by loss of a water molecule is shown in the following:

```
     H   O            H   O             H  O    H   O
     |  //            |  //             |  ||   |  //
+H3N-C-C     +  +H3N-C-C    --->  +H3N-C--C--N--C-C     + H2O
     |  \             |  \              |      | |  \
     R1  O-           R2  O-            R1     H R2  O-
```

Peptide bone

Many amino acids. usually more than 10 are joined by peptide bonds to form a polypeptide chain which is unbranched structure.

An amino acid unit in a polypeptide chain is called a residue.



Aminoterrninal Residue → Carboxyl Terminal Residue

A pentapeptide, the constituent amino acid residues are outlined.

The chain starts at the amino end.

A polypeptide chain has direction because its building blocks have different ends - namely, the α-amino and the α-carboxyl groups.

The sequence of amino acids in a polypeptide chain is written starting with the amino- terminal residue. Thus, in the tripeptide alanine-glycine-tryptophan, alanine is the amino-terminal residue and tryptophan is the carboxyl-terminal residue.

Note that tryptophanglycine-alanine is a different tripeptide.

A polypeptide chain is made up of a requlарly repeating backbone and distinctive side chains (R_1, R_2, R_3).

$$\begin{array}{c} HOHOHO \\ N-\overset{|}{C}-\overset{\|}{C}-N-\overset{|}{C}-\overset{\|}{C}-N-\overset{|}{C}-\overset{\|}{C}-H \\ HR_1HR_2HR_3 \end{array}$$

Many proteins such as myoglobin, consist of a single polypeptide chain. Others contain two or more chains which may be identical or different, for example, haemaglobin is made up of four chains: two chains of one kind and the other two of another kind.

Protein functions:

Proteins perform many essential functions in mammalian body. These functions may be grouped in two classes.

I. Dynamic functions: That include:

1. Transport: *i.e.* carrying important substances through the blood stream. *e.g.* Lipoproteins, hemoglobin and albumin.
2. Metabolic control: This function is performed by specific protein called enzymes that enhance the rate of chemical transformations inside the body.
3. Contraction: This is seen *e.g.* by proteins of muscles, myosin and actin, that slide in coordinate motion to produce muscular contraction, movement of chromosomes during mitosis and movement of sperms.
4. Protective: Immunoglobulins are specific proteins that act against invasion by bacteria or any other injurious agent. Fibrin products the body against blood loss by formation of the blood clot. Mucin that lines the respiratory and gastrointestinal tracts protects these regions against injuries and infection.

5. Regulatory function of proteins is done *e.g.* by hormones that regulate the rate of growth and differentiation of the body such as growth hormone, insulin, glucagen .. etc.

II. Structural functions of proteins include:

1. Mechanical support: Collagen and elastin make the matrix of bone and ligaments. They provide structural strength and elasticity. Keratin has an essential structural role in the epidermal tissue forming skin, hair and nails.
2. Generation and transmission of nerve impulse: *e.g.* Rhodopsin is a photoreceptor protein present in the retinal rod cell, it transmits the nerve impulse when it is bleached by light. Other cell receptors present on cell surface or inside the cells are also proteins.

Classification of Proteins

The proteins are classified mainly on the basis of their solubility, shape, function, physical properties and 3-dimensional structure.

Solubility:

Classification of proteins based on their solubilities:

Albumin: ⟶ Soluble in water and a salt solutions.

Globulins: ⟶ Sparingly soluble in water, but soluble in salt solution.

Protamines: ⟶ Soluble in 70-30% ethanol, but insoluble in water and absolute ethanol.

Histones: ⟶ Soluble in salt solution.

Scleroproteins: ⟶ Insoluble in water or salt solutions.

Overall shape:

Two broad classes of proteins may be distinguished:

1. **Globular proteins:** are characterised by compactly folded and coiled polypeptide chains. Examples include insulin, plasma albumine and globulins and many enzymes.
2. **Fibrous proteins:** are characterised by polypeptide chains or groups of chains coiled in a spiral or helix and cross-linked by disulfide and hydrogen bonds. Examples include keratin (the major protein of hair, wool and skin) and myosin (the major contractile protein of muscle).

Function:

Proteins may be classified according to their biological functions for example, as structural, catalytic (e.g. enzymes) or transport proteins (e.g. plasma lipoproteins).

Three - dimentional structure:

Two broad classes of proteins may be distinguished on the basis of whether or not they possess quaternary structure.

Another classification of proteins:

A. Simple proteins:

Which yield on complete hydrolysis chiefly amino acids: –

1. Protamines:

These have small molecular weight (2000) and contain only about eight different amino acids. They contain a large amount of arginine and so they are strongly basic and can absorb C02 from the air. They occur in sperm cells and are found in association with nucleic acids in nucleoproteins.

2. Histones:

They are also strongly basic proteins occurring as part of the nucleoproteins. The protein part of haemoglobin, globin, is a typical histone having a high content of histidine and lysine.

3. Albumins and globulins: (Coagulable proteins)

These are typical proteins. They contain most of the amino acids. Albumins are soluble in water and salt solutions. Globulins are sparingly soluble in water but soluble in salt solutions. Both are coagulated by heat. They are mostly found together in milk and egg-white. Plasma contains at least four globulins: fibrinogen, and a, β and y globulins. They are also widely distributed in plants especially in the seeds and fruits. Globulins are (salted out) in half-saturated ammonium sulphate solution, while albumins are salted out of solution by full saturation with ammonium sulphate. The two groups can be separated on this base.

4. Gliadins and glutelins:

– These are plant proteins, peculiar to the seeds of cereals. Both are insoluble in water but soluble in very dilute alkalis and acids. Gliadins can be separated from gluteliris by means of 70% alcohol in which gliandins only are soluble.

– Gliadin is the main protein in white flour (wheat). It is nutritionally a poor protein because of its deficiency in lysine, one of the essential amino acids. Corn contains a gliadin known as Zein which is low in tryptophan and lysine Wheat contains also glutelin.

5. Scleroproteins: (Albuminoids)

These are similar to albumins and globulins but they are insoluble in water and salt solutions. They form most of the cartilage and white fibres of connective tissue, elastin in the yellow or elastic fibres, ossein in bones and teeth, and keratins in hair, Wool, real silk and feathers. Thee keratins differ from the others in having an abnormally high sulphur content, chiefly in the form of cystine.

B- Conjugated proteins:

They are proteins that contain a non-protein prosthetic group beside its amino acid content.

1. Phosphoproteins:

These are proteins which have phosphoric acid as a prosthetic group. The phosphoric acid is esterified through the hydroxyl groups of serine and threonine. Caseinogen found in milk and vitellin found in egg-yolk, are phosphoproteins. Caseinogen is present in milk as the calcium salt. It is precipitated by acidification to its isoelectric point (pH 4.7). The enzyme rennin transforms caseinogen to casein and a peptide. The casein is soluble and is precipitated as calcium caseinate and thus the milk will clot.

2. Glycoproteins:

The prosthetic, group of glycoproteins consist of one or more heterosaccharide units attached to the polypeptide chain. The carbohydrate side chains consist of a small number (compared with mucopolysaccharide) of sugar residues.

3. Nucleoproteins:

These are compounds of proteins with nucleic acids. They are found is all cell nuclei and the protoplasm of cells. The protein part may be a histone or a protamine. The chromatin of nucleus contains desoxyribonucleic acid (DNA). The ribonucleic acid (RNA) occurs chiefly outside the nucleus on the cytoplasm.

4. Chromoproteins:

These are proteins with a coloured prosthetic group. Important members of this group are, the haemoglobin, cytochromes, flavo proteins and visual purple.

5. Lipoproteins:

These are proteins united with lipids. They occur in blood serum, in brain tissue, eggs. The cell membrane is of liporotein structure.

III- Derived proteins:

These are partially hydrolysed proteins:-

1- **Metalloproteins:**

The most complex of these products, are described as acid or alkali metaproteins according to as acid or alkali is used for hydrolysis.

2- **Proteoses:**

The next simpler hydrolysis products are called proteoses. They are more soluble and can be precipitated by saturated salt solutions. Gelatin is a proteose of collagen.

3- **Peptones:**

These are still simpler and not precipitated by saturated salt solutions.

Bonds Responsible for Protein Structure

Protein structure is generally stabilised by 2 classes of strong bonds (peptide and disulfide) and 3 classes of weak bonds (hydrogen. hydrophobic and electrostatic or salt).

1. Peptide bonds:

The peptide bond couples the α-carboxyl group of one amino acid residue to the α-amino group of another residue. There is no freedom of rotation about the bond between the carbonyl group atom and the nitrogen atom of the peptide group because

this link has partial double-bond character. There is by contrast, a large degree of freedom of rotation about the remaining bonds of the polypepUde backbone. This semirigidity has important consequences for orders of protein structures above the primary level.

Rigid unit Rigid unit

There is considerable freedom of rotation about the bonds joining the peptide groups to the carbon atoms.

2. Disulfide bonds:

The disulfide bond formed between 2 cysteine residues interconnects protein chains through cysteine residues This relatively stable cystine bond is resistant to usual conditions for protein denaturation.

Two peptide chains united by a disulfide linkage.

3. Hydrogen bonds:

Hydrogen bonds formed between bonding residues present in the side chains of peptide-linked amino acids and these formed

between the hydrogen and oxygen atoms of the peptide bonds themselves, all play important roles in the maintenance of protein structure above the primary order.

```
         R
         |
    —C—C—N—
     ||  |  |
     O   H  H
    *:      :*
     H   H  O
     |   |  ||
    —N—C—C—
         |
         R
```

* Hydrogen bonds.

4. Hydrophopic interactions:

— The nonpolar side chains of neutral amino acids tend to be closely associated with one another in proteins. No true bond may be said to exist. Nonetheless, these interactions play significant role in maintaining protein structure. The bonds are weak and disrupted by heat or by competition for another hydrophobic compound as a chloroform.

— Hydrophobic bonds are probably the most important for the determination of tertiary structure.

5. Electrostatic bonds:

These are salt bonds formed between oppositely charged groups in the side chains of amino acids. The epsilon-amino group of lysine bears a net charge of + 1 at physiologic pH and the non-a-carbonyl of aspartate and glutamate bear a net charge of -1. These may therefore interact electrostatistically to stabilize protein structure.

Bond stabilities:

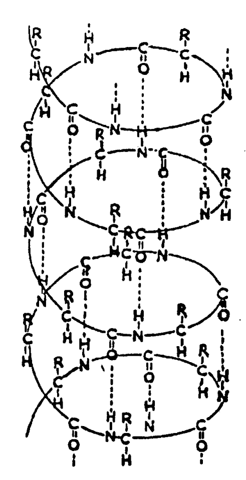
During denaturation of proteins, hydrogen, hydrophoblic and electrostatic bonds - but not peptide or disulfide bonds are broken.

Ordered Conformations of Polypeptides (Periodic Structures)

The α-Helix:

The a-helix is a rod like structure. The tightly coiled polypeptide main chain forms the inner part of the rod$_1$ and the side chains extend outwards in hydrogen bonds between the NH and CO groups of the main chain. The CO group of each amino acid is hydrogen bonded to the NH group of the amino acid that is situated forth in position in the linear sequence e.g. amino acid number one is attached to amino acid number 4 and number 2 is attached to number 5 and so-on

on.



Alpha helix structure of a protein

β-pleated sheet:

It differs from the α-helix in that it is a sheet rather than a rod. The polypeptide chain in the β-pleated sheet is almost fully extended rather being tightly coiled as in the α-helix. Another difference is that the β-pleated sheet is stabilized by hydrogen bonds between NH and CO groups in different polypeptide strands, while in the α-helix the hydrogen bonds are between NH and CO groups in the same polypeptide chain. Extensive regions of the β-pleated sheet structure have been found only in silk fibres. However, short segments of the polypeptide backbone of a number of proteins are arranged in a conformation similar to that of the β - pleated sheets.

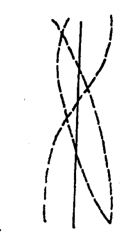
Diagrammatic representation of formation of a region of pleated sheet structure by formation or hydrogen bonds (...) between 2 regions or polypeptide chain. The bond angles are such that, when viewed on end, the polypeptide chains assume a conformation resembling a pleated sheet or paper.

Collagen helix:

Collagen is a major type of fibrous protein present in higher animals making up one-third or more of the total body protein. Although collagens of different species differ somewhat in amino acid sequences, most contain about 35% glycine and 11% alanine. Collagens are distinctive in containing about 12% proline and 9% hydroxyproline₁.an amino acid rarely found in proteins rather than collagen.

The secondary structure of collagen is that of a triple helix of polypeptide chains. Each of the chains is a left- hand three residue helix. The chains are held together by hydrogen bonds. The frequent proline residues determine the distinctive type of helical arrangement of the chain, while the smaller R group of the glycine residues, which occur in every third position, allow the chain to intertwine.

No proteins other than collagens appear to contain similar triple-helical chains.



Three stranded tropocollagen molecule.

Orders "Levels" of Protein Structure

Primary structure:

— The primary structure of protein is defined as a linear polypeptide chain that is composed of amino acid residues linked together through peptide bonds.

— When the number, chemical structure, order of all amino acid residues and the location of disulfide bridges, if present, are known, the primary structure is then determined.

— The primary structure is the simplest level of organisation of any protein molecule.

Secondary structure:

— The folding of polypeptide chain into a specific coiled structure held together by disulfide bonds and by hydrogen bonds is referred to as the "secondary structure" of the protein

— The secondary structure refers to the steric relationship of amino acid residues that are close to one another in the linear sequence of the polypeptide chain.

— α-helix and β-pleated sheet are examples of secondary structure.

Tertiary structure:

— Is the interaction between several domains or far apart amino acids of the same polypeptide chain. This results in appearance of a three dimensional structure. The tertiary structure is maintained by weak forces e.g. hydrogen bonds.

— The shape of protein at this level of organization (its conformation) is responsible for its biological function e.g. fibrous proteins (proteins forming fibres and membranes) will be consisted of coiled ropes of peptide chain, while globular proteins (having roughly spherical) conformation are formed by compact folding of the peptide chains upon themselves.

Quanternary structure:

Proteins are said to posses quaternary structure if they consist of 2 or more polypeptide chains united by forces other than covalent bonds (i.e. not peptide or disulfide bonds). The forces that stabilize these aggregates are hydrogen bonds and electrostatic (or salt) bonds formed between residues on the surfaces of the polypeptide chains, such proteins are termed oligomers and the individual polypeptide chains of which they are composed are variously termed protomers monomers, or subunits.

— The most commonly encountered oligomeric proteins contain 2 or 4 protomers and are termed dimers or tetramers, respectively.

— Oligomeric proteins play special roles in intracellular regulation, because the protomers can assume different spatial orientation relations to each other with resulting changes in the properties of the oligomer. The best example is haemoglobin, in which a variety of conformations exist depending on the degree of oxygenation.

Properties of proteins

Pure proteins are generally tasteless, odourless and soluble in different solvents according to which they are classified.

Molecular weight and colloidal properties:

1. Protein molecules are exceedingly complex in structure and very large. Their molecular weight ranges from ten thousands to many millions.
2. Because of their large size they form colloidal solutions of emulsoid type or hydrophilic colloids which are stable in solution due to the charge and hydration film surrounding the particles. At the isoelectric point the particles are acid or alkaline side of it, they carry positive and negative charges respectively.
3. In addition the large molecules do not diffuse through semipereable membranes and hence can be dialyzed from inorganic salts.

Reactions of proteins:

Although many of the NH_2 + COOH groups of the constituent amino acids have been neutralized in the formation of

peptide linkages, there will always be some free groups either from the ends of the peptide chains or the excess groups of acidic and basic amino acids. Proteins with equal free amino and carboxyl groups are neutral in reaction, but those which show more free amino groups are basic in reaction as protamines whereas proteins with more free COOH groups are acidic in reaction as gliadins.

Amphoterism, Zwitterion and Isoelectric Point:

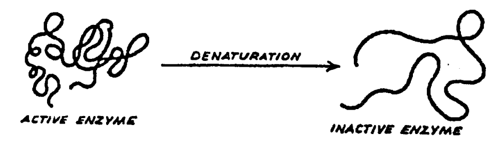
— The proteins like amino acids are amphoteric, dipolar ions (Zwitterions) and can combine with both acids and bases forming salts which ionize into a colloidal protein ion and one crystalloidal ion (see explanation under amino acids using NH_2-R-COOH to represent the protein molecule).

— In solutions acid to their isoelecteric points proteins exist as positively charged ion capable of combining with negative ions to form salts while in solutions alkaline to their isoelectric point proteins exist as negatively charged ions which can combine only with positive ions.

— At the isoelectric point the solubility of the protein is reduced and is easily precipitated from solutions, also other physical properties of proteins as viscosity, osmotic pressure are at a minimum.

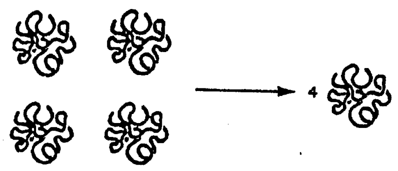
Denaturation:

The comparatively weak forces responsible for maintaining secondary, tertiary and quaternery structure of proteins are readily disrupted by a variety, of manipulations with a resulting loss of biologic activity. This disruption of native structure is termed denaturation. Physically, denaturation may be viewed as an altered conformation of a polypeptide chain without affecting its primary structure. For protomer, the process may be represented as shown:



Representation of denaturation of a protein.

— For an oligomeric protein, denaturation may involve dissociation of the protomers without accompanying changes in protomer conformation.



Representation of denaturation of an oligometric protein under conditions not sufficiently severe to alter protomer conformation

— The biologic activity of most proteins is destroyed by exposure to strong mineral acids or bases, heat, ionic detergents, urea, heavy metals (Ag, Pb, Hg) or organic solvents at or above room temperature.

The usual changes in native protein when denatured:

1. Decreased solubility, diffusion and increased iscosity of the protein solution.
2. Increased digestibility by proteolytic enzymes where the internal peptide bonds are exposed by opening up of the protein structure either by swelling or unfolding.
3. Loss of enzymatic properties if the protein is an enzyme.

4. Exposure of oxidizing and reducing groups especially –SH groups due to unfolding of polypeptide chains.
5. Modification of antigenic properties.

Separatory techniques for proteins

If there is mixture of proteins, they can be separated by different ways that are based on one more of the following basis:

a) Solubility of the protein needed to separated, *e.g.* salting out.
b) Size of the protein molecule, *e.g.* dialysis, some types of chromatography and ultracentrifugation.
c) Charge of the molecule, *e.g.* electrophoresis.

1) Salting out:

This method is based on elimination of water from the solution *e.g.* albumin is separated at a fully saturated solution and globulin is separated at ½ saturated solution of ammonium sulfate.

2) Ultracentrifugation:

This is done by putting the mixture in a high speed centrifuge. It is used to separate lipoproteins from other plasma proteins. Lipoproteins will separates into layers according to their protein contents, molecular weight and density.

Dialysis:

Proteins can be separated from small molecules by dialysis through a semi permeable membrane. Molecule with a molecular weight greater than about 15,000 are retained inside a typical dialysis bag whereas smaller molecules and ions traverse the pores of such a dialysis membrane and emerge in the dialysate outside the bag.

Gel-fitration chromatography:

Here the separation also depends on the size of the molecule. The sample is applied to the top of a column consisting

of an insoluble but highly hydrated carbohydrate polymer in the form of beads, which are typically 0.1 mm is diameter. Sephadex is a commonly used commerical preparation. Small molecules can enter these beads, but large ones can not. The result is that small molecules are distributed in the aqueous solution inside the beads and also between them, whereas large molecules are located only in the solution between the beads. Large molecules flow more rapidly through this columin and emerge first because a smaller volume is assessible to them.

Ion-exchange chromoatography:

Proteins can also be separated on the basis of their net charge by ion-exchange chromatography. If a protein has a net positive charge at pH 7, it will usually bind to an ion- exchange column containing carboxylate groupse whereas a negatively charged will not. Such a positively charged protein can be released from the column by adding NaCl or another salt to the eluting buffer. Sodium ions compete with positively charged groups on the protein for binding to the colum in proteins that have a low density of net positive charge will tend to emerge first followed by those having a higher charge density.

Factors other than net charge also influence the behaviour of protein on ion -exchange columns.

Electrophoresis:

– Electrophoresis is the migration of charged particles in an electrolyte solution which occurs when an electric current is passed through the solution.

– Various protein components of a mixture, as plasma, at pH values above and below their isoelectric points will migrate at varying rates in such a solution because they possess different surface charges.

Nucleoproteins and Nucleic acids

Nucleoproteins are one of the conjugated proteins they are formed of non-protein prosthetic group (nucleic acid) attached to one or more molecules of a simple protein. This simple protein is usually basic protein as histone or protamine. These conjugated proteins are found in all animal and plant tissues but are most easily isolated from yeast or from cells whose nuclei are densely packed e.g. thymus cells.

Biomedical importance:

1- The heterocyclic bases purines and pyrimidines are the parent molecules of nucleotides which are present in every cell performing numerous key functions.

2- Repeated units of nucleotides form nucleic acids either DNA or RNA. DNA is present in the nucleus forming chromosomes which are responsible for transmission of genetic characters including regulation of protein synthesis. RNA is present in both nucleus and cytoplasm and has a central role in the process of protein synthesis.

3- Nucleosides triphosphate e.g. ATP, GTP, ... etc play an important role in energy transduction.

4- Nucleosides monophosphate may act as coenzymes e.g. AMP, regulator of enzymatic activities, and second messengers e.g. cyclic AMP (cAMP) and cyclic GMP (cGMP).

5- Structure of viruses is almost nucleoproteins.

6- Syntheic analogs of normally occurring nucleotides may find application in the treatment of cancer. These analogs can act as enzyme inhibitors or replace the natural nucleotides in nucleic acids synthesis in this way viral growth and cancer cell replication are inhibited.

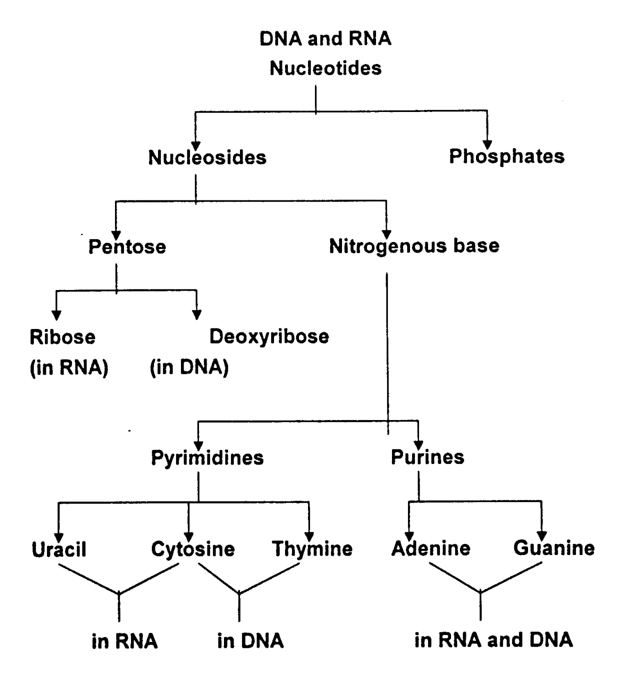
Structure of nucleoproteins

As mentioned acid hydrolysis of nucleoproteins yields a protein part (histone or protamine) and non-protein part, nucleic acids.

Nucleic acids:

- Nucleic acids can be hydrolyzed either by acids or use of enzymes producing a number of nucleotides. Nucleic acids are therefore polynucleotides.
- Each nucleotide can be further hydrolyzed into nucleoside and phosphoric acid molecule.
- Further hydrolysis of nucleosides yields a nitrogenous base (purine or pyrimidine) in addition to pentose sugar (ribose or deoxy ribose) as can be seen the diagrammatic representation.

NUCLEIC ACIDS



PURINES AND PYRIMIDINES

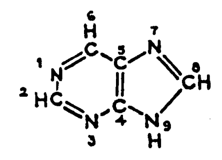
They are important cellular molecules of low molecular weight that participate in many chemical processes. They all contain nitrogen so they are called nitrogenous bases.

Purines:

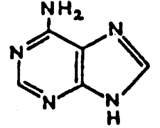
The purines serve as:-

* Monomeric precursors of nucleic acids (DNA and RNA).
* Precursors of high energy sources *e.g.* ATP.
* Precursors of regulatory signals *e.g.* cyclic AMP.
* Component of coenzymes, FAD, NAD and NADP.
* Component of the methyl donor, S-adenosylmethionine.

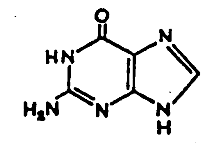
The purine bases, adenine and guanine are the two major purines found in living organisms.



Purine



Adenine
(6-aminopurine)



Guanine
(2-amino-6-oxypurine)

Xanthine and hypoxanthine occur as intermediates in the metabolism of adenine and guanine.

Hypoxanthine (6-oxypurine)

Xanthine (2,6-dioxypurine)

Uric add is formed as the end product of purina catabolism.

Uric acid

Purines are biologically important molecules which participate in a wide variety of biochemical processes of the body. They are components of:

- Purine nucleotides servings as monomeric precursors of nucleic acids (RNA and DNA).
- Purine nucleotides serving as high energy phosphate compounds in the cell e.g. ATP and GTP.
- Intracellular chemical mediators of hormonal action e.g. cyclic AMP and cyclic GMP.
- Purine nucleotides serving as functional moieties of some coenezymes e.g. FAD, NAD^+, $NADP^+$ and coenzyme A.

- The methyl donor, S-adenosyl methionine.
- The sulfur donor, 3`-phosphoadenosine-5`-phospho-sulphate (PAPS).
- Certain synthetic purines have important applications e.g. 6-mercaptopurine used as a chemotherapy of cancer.

— In plants a series of purino bases containing methyl substituent occur e.g. coffee which contains caffeine. tea which contains theophylline and cocca contains theobromine.

Caffeine (1,3,7-trimethyl-xanthine)

Theophylline (1,3-dimethyl-xanthine)

Theobromine (3,7-dimethyl-xanthine)

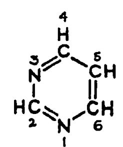
Pyrimidines:

They are present in nucleic acids and serve as high energy intermediates *e.g.* UDP-glucose and UDP-galactose. Examples of pyrimidine derivatives found in nucleic acids include uracil, thymine and cytosine.

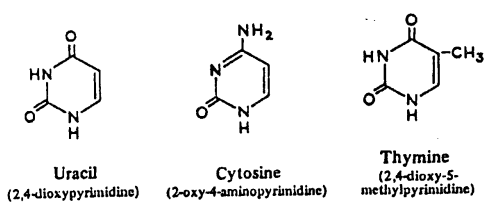
Pyrimidine bases are formed of heterocyclic rings containing nitrogen, so they are called nitrogenous bases. In humans, there are 3 major pyrimidine bases found in the nucleotides forming the monomeric units of nucleic acids, they are:

1. Uracil (2, 4-dioxypyrimidine).
2. Cytosine (2-oxy-4-amino pyrimidine).
3. Thymine (2, 4-dioxy-5-methylpyrimidine = 5-Methyluracil)

Uracil is found only in RNA, thymine in DNA, while cytosine is found in both RNA and DNA.



Pyrimidine



Uracil (2,4-dioxypyrimidine) **Cytosine** (2-oxy-4-aminopyrimidine) **Thymine** (2,4-dioxy-5-methylpyrimidine)

- Pyrimidine nucleotides serving as monomeric precursors of nucleic acids (RNA and DNA).
- Pyrimidine nucleotides serving as high energy phosphate compounds in the cell e.g. UTP and CTP.
- Intermediates in some pathways of carbohydrate metabolism e.g. UDP-glucose used in synthesis of glycogen, lactose and glucuronic acid.

- Intermediates in some pathways of lipid metabolism e.g. CDP-choline used in synthesis of phosphatidyl choline and sphingomyelin.
- Vitamin B_1 (thiamine) which contains a pyrimidine derivative in the form of 2, 5-dimethyl-6-amino pyrimidine.
- Certain synthetic pyrimidines have important biological applications:
  - Alloxan (2, 4, 5-6-tetraoxypyrimidine) produces experimental diabetes in animals.
  - Thiouracil and related compounds are used in the treatment of hyperthyroidism.
  - 5-fluorouracil is used as a chemotherapy of cancer.

NUCLEOSIDES

In nucleosides the bases are linked to a pentose sugar in a β-D configuration. For example, the structures of aderosine, guanosine, uridine, and cytidine are represented as follow:

Adenosine

Guanosine

Uridine

Cytidine

The pentose sugar is present as β-ribose in ribonudeic acid (RNA) and as β-deoxyribose in deoxyribonucleic acid (DNA). They are attached N-glycoside linked to N_6 of purine or N_1 of pyrimidine.

Thymidine Deoxyguanosine

In ribonucleosides, a purine or a pyrimidine base is linked to a ribose sugar. Thus, the ribonucleoside adenosine consists of adenine with ribose sugar attached at its N_9 position. Gunosine consists of guanine with ribose attached at its N_9 position. Cytidine is cytosine with ribose attached at its N_1 position. Uridine consists of ribose attached at the N_1 position of uracil. Adenosine, guanosine, cytidine and uridine are given the abbreviations A, G, C and U, respectively.

The 2`-deoxyribonucleosides consist of 2-deoxyribose attached to the purine or pyrimidine bases guanine, cytosine and thymine deoxyribonucleosides are called deoxyadenosine, deoxyguanosine, deoxycytidine and thymidine. They are given the abbreviations dA, dG, dC and T, respectively. The prefix "d" is added to denote that the sugar of the nucleoside is 2-deoxyribose.

The sugar commonly found attached to thymine is 2-deoxyribose and that commonly found attached to uracil is ribose. Therefore, thymidine (T) consists of thymine + 2-deoxyribose while uridine (U) consists of uracil + ribose.

Nucleosides are hydrolytic products of nucleic acids and nucleotides, and usually present free.

Nomenuclature of nucleosides is shown in the following table.

Base	Ribonucleoside	Deoxyribonucleoside
Adenine	Adenosine	Deoxyadenosine
Guanine	Guanosine	Deoxyguanosine
Cytosine	Cytidine	Deoxycytidine
Uracil	Uridine	Deoxyuridine
Thymine	Thymine riboside	Thymidine

NUCLEOTIDES

The hydrolysis of nucleic acids yields mono-nucleotides. The mononucleotide contains a nitrogenous base, a sugar, in addition to phosphoric acid. In other words, nucleotides are phosphorylated nucleosides on one or more of the hydroxy groups of the sugar.

Nomenclature of nucleosides is shown in the following table

Base	Ribonucleosides
* Adenine	Adenylic acid, Adenosine-5`-monophosphate (AMP)
* Guanine	Guanylic acid, Guanosine-5`-monophosphate (GMP)
* Xanthine	Xanthylic acid, Xanthosine-5`-monophosphate (XMP)
* Hypoxanthine	Inosinic acid, Inosine-5`-monophosphate (IMP)
* Cytosine	Cytidylic acid, Cytidine-5`-monophosphate (CMP)
* Uracil	Uridylic acid, Uridine-5`-monophosphate (UMP)

Nomenclature of deoxyribonucleotides is shown in the following table

Base	Ribonucleosides
* Adenine	Deoxyadenylic acid, Deoxyadenosine -5`-monophosphate (dAMP)
* Guanine	Dexyguanylic acid, Deoxyguanosine-5`-monophosphate (dGMP)
* Cytosine	Deoxycytidylic acid, Deoxycytidine-5`-monophosphate (dCMP)
* Thymine	Thymidylic acid, Thymidine-5`-monophosphate (TMP)
* Uracil	Uridylic acid, Uridine-5`-monophosphate (UMP)

In transfer RNA (tRNA) the ribose moiety is occasionally attached to uracil at the 5 position forming a carbon to carbon linkage instead of the usual nitrogen to carbon linkage producing pseudouridine.

Naturally occurring nucleotides:

Free nucleotides may be part of nucleic acids or may be found free in tissues to perform certain functions.

I. Adenosine derivatives:

1) Adenosine monophosphate (AMP = adenylic acid) is a component of nucleic acids.
2) Adenosine diphosphate (ADP) and adenosine triphosphate (ATP) are participants of oxidative phorphorylation (cellular respiration). ATP is the most abundant intracellular nucleotide and is a source of high energy phosphate for most energy requiring reactions in the cell.
3) Cyclic AMP = 3',5' cyclic adenosine monophosphate (CAMP): It acts as a mediator of extracellular signals e.g. it acts as a secondary messenger for the action of hormones.
4) S-adenosylmethionine serves as a form of active methiotilne that acts as a methyl donor in many methylation reactions.
5) 3'-phosphoadenosine-5'-phosphosulfate CPAPS) acts as active sulfate used, for example$_3$ in the synthesis of sulfated mucopolysaccharides.

CYCLIC 3',5'-AMP

Adenine

Ribose

Adenosine 5'-monophosphate (AMP)

Adenosine 5'-diphosphate (ADP)

Adenosine 5'-triphosphate (ATP)

METHIONINE

ADENOSINE

The structure of S-adenosylmethionine

3'-phosphoadenosine-5'-phosphosulphate (PAPS)

II. Guanosine derivatives:

1) Guanosine monophosphate (GMP) = Guanylic acid is a component of nucleic acids.
2) Guanosine diphosphate and triphosphate (GDP and GTP) serve as high energy phosphate compounds as ADP and ATP in some cellular reactions.
3) Cyclic OMP (3',5' CGMP) is an intracellular signal for some extracellular events e.g. some hormones.

III. Uracil derivatives:

1) They are important coenzymes in metablism of hexoses.
2) They serve as hexose donors in polymerization of sugars to form e.g. glycogen and glycoproteins. In these reactions UDP is the sugar carrier e.g. UDP-glucose (UDPG) or UDP-galactose (UDP Gal).
3) UTP may serve as a high energy phosphate for some reactions in the cell, and as a source of uridine nucleotides in RNA.
4) UMP = uridylic acid is a component of RNk.

IV. Cytosine derivatives:

1) Cytidine triphosphate (CTP) is a precursor for CMP units of the nucleic acids.
2) CDP-choline donates choline to ceramide to yield sphingomyelin.
3) Cyclic CMP is analogous to cAMP and cGMP.
4) CMP = cytidylic acid is a component of nucleic acids.

V. Thymine derivatives:

1) Thymidylic acid (TMP) is a component of DNA.
2) TDP and UP are high energy phosphate compounds intracellularly.

VI. Vitamin nucleotides:

Some of the β-complex vitamins serve as constituents of coenzymes with functional moieties in the form of nucleotides e.g. riboflavin is a constituent of FAD, niacin is a constituent of NAD and NADP and panthothenic acid is a constituent of coenzyme A. These coenzymes will be discussed later in vitamins.

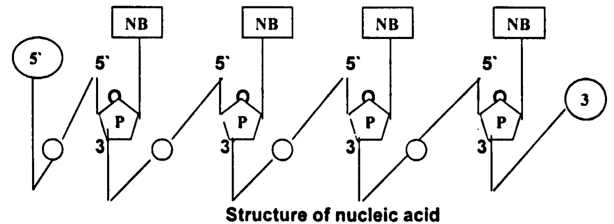
NUCLEIC ACIDS

Structure of nucleic acids:

— Nucleic acids are long chain of repeating subunits of mononucleotides linked together by 3`-5`-phosphodiester bonds.

— The 3`-5`-phosphodiester bond is made by esterification of the phosphate group of C_5 of the pentose of each nucleotide to the OH group of C_3 of the pentose of the adjacent nucleotide. So, the phosphate group is attached by two ester bonds to carbons No. 3` and 5` of two pentoses of two successive nucleotides.

The 3`, 5`-phosphodiester bonds will content together the successive mononucleotides through the whole length of the nucleic acid polymer. The resultant polynucleotide chain will possess a polarity; one end has a 5`-phosphate or hydroxyl terminus while the other has a 3`-hydroxyl or phosphate moiety.



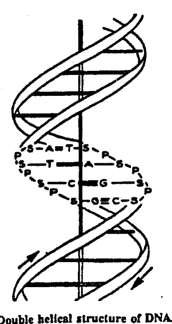
Structure of nucleic acid

NB = nitrogenous base P = pentose (P) = phosphate

Deoxyribonucleic acid (DNA) is present in animal cells mostly in the nucleus, whereas most of ribonucleic acid (RNA) is present in the cytoplasm.

DNA

- Nuclear DNA is the major constituent of the chromosome It exists as a thin double helix. The double helix is folded and complexed with protein to form the chromosomal strands.
- The two strands of this right-handed double stranded molecule are held together by hydrogen bonds between the purine and pyrimidine bases.
- The pairing between the purine and pyrimidine nucleotides on the opposite strands are very specific (complementary) and are dependent upon hydrogen bonding of adenine to thymine by two hydrogen bonds (A = T) and guanine with cytosine by three hydrogen bonds (G C).
- The 2 strands of the double helical molecule, are antiparallel i.e., one strand runs in the 5' to 3' direction and the other in the 3' to 5' direction.
- The backbone of double stranded helix is formed by the sugar (deoxyribosa) and phosphate whereas the bases are present in the inside of the molecule.



Double helical structure of DNA.

Functions of DNA:

The genetic information stored in the nucleotide sequence of DNA
serves 2 purposes: -

1) It is the source of information for the synthesis of all cell proteins.
2) It provides the information inherited by doughter cells (offsprings).

Properties of DNA:

1) Heating of DNA with strong acid hydrolyses it to its constituents i.e. sugars, phosphate, purine and pyrimidine bases (C, T, A and G).
2) Enzymatic hydrolysis of DNA produces mono and oligo nucleotides.
3) Denaturation of DNA occurs due to crowdness of the bases. The native DNA molecule is viscous in solution, but when is denaturated it loses its viscosity.

Comparison between DNA and RNA

	DNA	RNA
1- Occurance	Chiefly in the nucleus	Chiefly the cytoplasm
2- Shape	Double helix	Single strand
3- Types	One type	Three types
4- Sugar	Deoxyribose	Ribose
5-Bases Purines Pyrimidines	 Adenine, guanine Cytosine, thymine	 Adenine, guanine Cytosine, uracil
6- Function	Forms the genes and direct synthesis of mRNA	Protein synthesis

RNA

Ribonucleic acid is a polymer of purina and pyrimidine nucleotides linked together by 3', 5' phosphodiester bridges as those in DNA. It differs from DNA in the following features:—

1) The sugar moiety attached to the nitrogenous bases is ribose (in DNA it is deoxyribose).
2) It contains adenine, guanine, cytosine and uracil (in DNA there is thymine instead of uracil).
3) It is single stranded (DNA is double stranded).
4) It can be hydrolysed by acid or alkali (DNA is hydrolysed by acid only).
5) It is present mainly in the cytoplasm (DNA is present in the nucleus). RNA is present also in the nucleus.

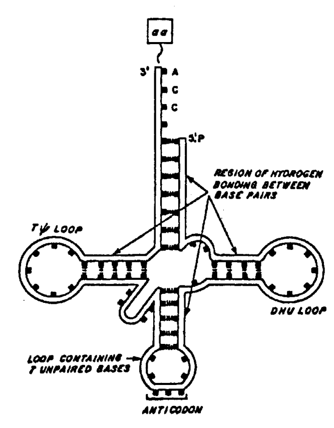
A segment of a ribonucleic acid (RNA) molecule in which the purina and pyrimidine bases-adenine (A), uracli (U), cytoeine (C), and guanine (G)-are held together by phosphodiester bonds between ribosyl moieties anached to the nucleobases by N glycosidic bands. Note that the polymer has a polarity.

Types of RNA:

There are three types of cellular RNA:

1) Transfer RNA (tRNA):

— It Is called also soluble RNA (sRNA). It acts to transfer active amino acids Into proteins. There is a specific tRNA for each amino acid. tRNA carries the amino acid on its 3' end and recognises its position on protein by its anticodon loop. So it acts as adaptor for the translauon of the information in the sqeuence of nucleotides of mRNA into specific amino acid.



Transrer RNA (tRNA). Note the site or attachment of the amino acid to the 3 terminus or IRNA.

2- Messenger RNA (mRNA):

— It constitutes 5% of all RNA. It is synthesized in the nucleus by DNA and then sent to the ribosomes in the cytoplasm.

— The mRNA carries a message from DNA in the nucleus, where it is synthesized, to the ribosomes in the cytoplasm, where it

is going to direct the synthesis of a specific protein. The letters of the message are the nitrogenous bases, the sequence of which is responsible for arranging the amino acids in proper order in the polypeptide chain to be synthesized.

— Each 3 successive bases in the mRNA are called a "codon" because they code for a specific amino acid.

— The process of synthesis of mRNA in the nucleus under the directions of DNA is called "transcription".

3- Ribosomal RNA (rRNA):

— It constitutes 80% of total RNA. It is present in the ribosomes of the cytoplasm.

— The ribosome is a cytoplasmic nucleoprotein structure which acts as the machinery for the synthesis of proteins. On the ribosomes, the mRNA and tRNA molecules interact to translate a specific protein.

— The ribosome particle is formed of at least four rRNA molecules complexed with several protein molecules.

— The presence of rRNA molecules as a part of the structure of the ribosomal particle helps in formation of its compact configuration which seems necessary for binding of mRNA to the ribosome in order to be translated.

— The ribosomal particle consists of 2 subunits. The larger subunit is known as 60S, while the smaller one is known as 40S (S = Svedberg unit, measured by ultracentrifugation).

IMMUNOCHEMISTRY

IMMUNOCHEMISTRY

Introduction to Basic Immunology

Immunology is the science which studies the immunity.

Immunity is the resistance of the body against infection i.e. it is the reaction of the body against any foreign materials introduced into the body such as bacteria, viruses, protozoa and toxins.

Lines of body defence against infection:

There are three lines:

1. Skin and mucous membrane:

They act as mechanical barriers for infection. Intact skin and mucous membrane is not easily penetrated by microorganisms.

2. Secretions:

— Saliva, gastric, intestinal, nasal, bronchial secretions can dissolve and kill microorganisms.

— Also, tears and sweating have anti-microbial effect.

— Furthermore, the act of sneezing, coughing and vomiting will get rid of the microorganisms.

3. Phagocytic cells:

— If the foreign materials overcome the first and second lines of defence. They will be taken by phagocytic cells and fragmented into small fragments (antigenic materials).

— The body then can recognize these antigens as foreign materials and react against these by what is called *immune response*.

In order to understand the immune response we must know the immune system and the cells of immune response.

The Immune System:

A. Central organs: Bone marrow, thymus and bursa equivalents.

B. Peripheral organs: Spleen, lymph nodes and lymphocytes.

— The lymphocyte is the central immune cell.

— The bone marrow, thymus and bursa equivalents are important for its development and function.

— The stem cells of lymphocytes originate in the bone marrow and differentiate into 2 classes:

1. **T-lymphocytes** (thymus dependent lymphocytes):
   These T-lymphocytes are labeled and differentiated in the thymus to recognize body tissue proteins in order to avoid reaction with the body own proteins (i.e. to avoid autoimmune reaction).

2. **B-lymphocytes** (thymus independent lymphocytes):
   These B-lymphocytes are differentiated in the bone marrow and in the bursa equivalents in order to avoid reaction with the body own proteins.
   Both T-lymphocytes and B-lymphocytes after differentiation will migrate to the spleen and lymph nodes and settled there.
   T-lymphocytes are settled in the deep cortical area, while B-lymphocytes are settled in cortical area and inedullary cord.

N.B → Small parts of both T-lymphocytes and B-lymphocytes are present in the peripheral blood stream.

Notice

- On antigenic stimulation of T-lymphocytes they proliferate to form population of small T-lymphocytes (cell mediated immunity). A subpopulation of T-lymphomemory called T-helper, T-suppressor, killer and memory cells are formed and have an effect on immune regulation.

- On antigenic stimulation of B-lymphocytes, they proliferate and give rise to plasma cells that secrete immunoglobulins (antibodies).

Immune response:

– It is the response of the body against any foreign material (antigen) introduced into the body.

Steps of immune response:

1. Capture and processing of antigen:

– This is carried out by phagocytic cells (modified form of monocytes and poly morphonuclear leucocytes).

– Once the phagocytic cells have ingested the antigen it will be broken down into immunogenic fragments.

2. Antigen recognition:

– Both T- and B-lymphocytes are antigen reactive cells and capable of antigen recognition.

– The recognition of antigen is mediated by an antibody like molecule synthetized in the cell and bound to the surface membrane.

Types of immune response:

1. Cellular immune response:

– If T-lymphocytes recognize the antigen they react and proliferate to form colonies of small sensitized T-lymphocytes that sequestrate the antigen and secrete lymphokines and interferon.

– These sensitized lymphocytes mature to give T-helper and killer (cytotoxic),T-effector cells together with population of memory cells. This type of immune response is called *cellular immunity*.

Lymphokines:

They are produced from activated T-lymphocytes for regulation of immune response:

a) Interleukin-2: It allows the continued proliferation of antigen activated B and T lymphocytes.

b) Interferon : It is a protein named so because of its ability to interfere with intracellular virus replication. There are α, β and γ interferons.

γ interferon is the most potent. It stimulate IL-1 and IL-2 production. Also, it has inhibitory effect on cell growth.

2. Humoral immune response:

If B-lymphocytes recognize the antigen they react and proliferate to form colonies of plasma cells which secrete the immunoglobulins. This type of immune response is called humoral immunity.

Autoimmunity:

It is the condition in which the body produce antibodies against its own tissue proteins and often leads to tissue injury. This happens when self antigens are recognized as foreign by the individual.

Causes:

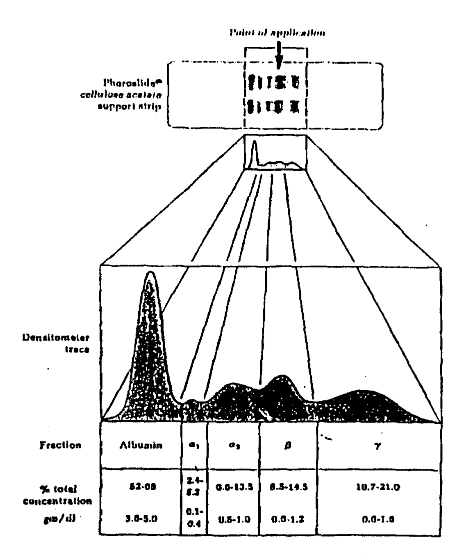
1. Drugs, chemicals, or infectious agents may alter normal body proteins to an antigenic form.
2. Foreign antigens that are antigenically related to body proteins produce antibodies which may cross-react with normal body proteins.
3. Defect in the development of immune recognition system due to the disease of the thymus in early life.

Examples of the autoimmune diseases:

- Systemic lupus erythematosis.
- Rheumatoid arthritis.
- Rheumatic fever. Addison's disease.
- Hashimoto's thyroiditis.

Immunoglobulins

They are class of plasma proteins secreted from plasma cells. For many years they were regarded as γ-globulins, the class of plasma proteins with the least electrophoretic mobility and they are called antibodies.



Protein electrophoretic separation of serum

— These antibodies arc able to bind specifically to the antigen to be inactivated or eliminated.

— Some antibodies remain bound to lymphocyte surfaces where they act as antigen receptors. There arc five classes of immunoglobulins known as IgG, IgA, IgM, IgD and IgE.

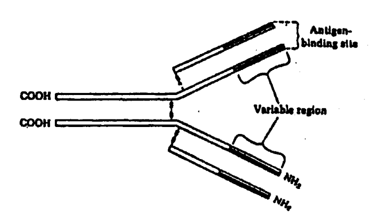
Strucatre:

— The basic structure of immunoglobulin molecules are the same and the molecule unit is composed of four polypeptide chains linked by disulphidc bonds, these arc two identical heavy chains (H-chain) and two identical light chains L-chains).

— The heavy chains are specific for the class of immunoglobulins.

— Differences in H-chain composition determine the class, that is γ chains for IgG, α chains for IgA, μ chains for IgM, δ-chain for IgD and ε chain for IgE.

— The two light chains have a lower molecular weight and are approximately one-half the length of the H-chains. L-chains are of two types, kappa (κ) and lambda (λ). An individual immunogiobulin molecule may be of type κ, having two κ chains or type λ having two λ chains.



Basic structure of IgG molecules

Both the heavy and the light chains can be divided into 2parts:-

1. Carboxy terminal half or crystalline fragment (Fc) in the heavy chains. This region have constant amino acid sequences and so it is called constant region. This crystalline fragment has certain biological importance.
   - Site for CRO binding.
   - Can pass the placenta.
   - Can fix complement.
   - Can be attached to the cell membrane.
   - Can not combine with antigen.
2. Amino terminal half; which has variation in amino acid sequcnces from molecule to molecule (variable region). This part of the molecule is the site of antigen binding and is called fragment of antigen binding (Fab).

Effect of enzymes on immunoglobulin molecule:

1. Papain:

Digestion of immunoglobulin molecule with papain result in 3 fragments as digestion occurs near the amino terminal end.

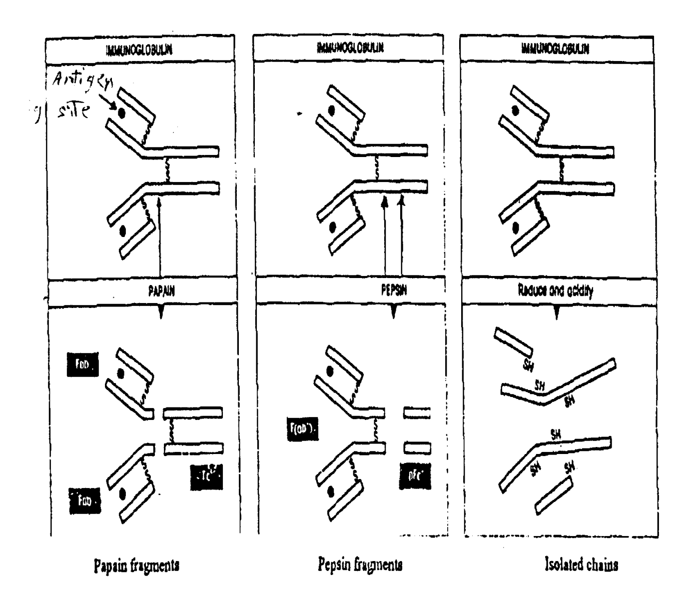
The three fragments are

- Antigen binding fragment 2 Fab.
- Crystalline fragment Fc.

2. Pepsin:

Digestion of immunoglobulin molecule with pepsin result in the formation of 2 fragments:

- Antigen binding fragment linked by disulphide bond $(Fab)_2$.
- Crystalline fragment that will be degraded to amino acids as digestion occurs near the carboxy terminal half.



Papain fragments Pepsin fragments Isolated chains

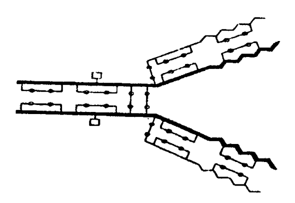
Classes of immunogobulins:

There are five classes of immunoglobulins differ in amino acid sequences in the constant region of the heavy chains.

	Immunoglobulins	H-chain	Symbol
1	IgG	Gamma	γ
2	IgA	Alpha	ά
3	IgM	Mu	µ
4	IgD	Delta	δ
5	IgE	Epsilon	ε

Immunoglobulin G:

- It is the predominant immunoglobulin in serum.
- It forms 75% of total Ig in serum 600-1600 mg/dl.
- It is present in monomer form and its heavy chain is called γ-chain.
- Its molecular weight is low, so it can pass the placental barrier and provide passive immunity for newborn in the first 2-6 months after delivery.
- It has a wide protective effect against bacteria, viruses, protozoa and toxins.
- It increases in secondary immune response.
- There are four subclasses IgG_1, IgG_2, IgG_3 and $1gG_4$ each has a distinctive heavy chain called γ_1, γ_2, γ_3 and γ_4.
- It can fix and activate complement.

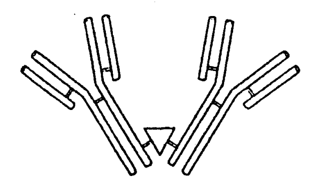


Structure of IgG

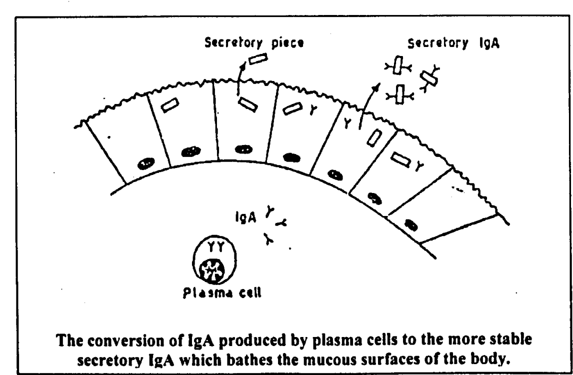
Immunoglobulin A:

- It is the second inununoglobulin as regard the concentration in serum. 200-500 mg/dl.
- It is present as monomer, dimer or trimer and its heavy chain is called α.
- It is the predorninant class of antibodies in the external secretions and therefore it is called secretory immuno-globulin.
- It is present in tears, saliva, nasal, bronchial and gastric secretions.

- Also, it is found in colostrum and milk and it is important for the body as it is not digested and absorbed intact to give immnunity to the body.
- It is present in high concentration in the form of dimer i.e. two molecules of IgA joined to each other by J-chain at the carboxy terminal ends. when they are passed through the epithelial cells, they are joined by secretory piece to prevent the digestion of IgA by proteolytic enzymes.
- It is responsible for local immunity.
- It has two subclasses IgA_1, and IgA_2 with a distinctive α_1 and α_2 chains.
- It can fix and activate the alternative pathway of complement.



IgA dimer



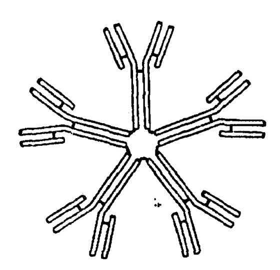
The conversion of IgA produced by plasma cells to the more stable secretory IgA which bathes the mucous surfaces of the body.

Inmunoglobulin M:

— Immunoglobulin M molecule consist of 5 units of IgG linked together to give pentamer shape. (star shape)

— The 5 units are linked together through the H-chains by J-chain which is the glue that stabilizes the multimeric Igs and gives the molecule star-fish appearance.

- Its concentration in serum 60-200 mg/dl and its heavy chain is μ chain.
- It is macroglobulin as it is composed of 5 units of IgG and so it can not pass the placental barriers.
- It is predominant in primary immune response.
- There are two subclasses IgM, and IgM_2 with a distinctive μ_1 and μ_2 chnins.
- It can fix and activate the classical pathway of complement.
- It gives agglutination reactions with the antigen.



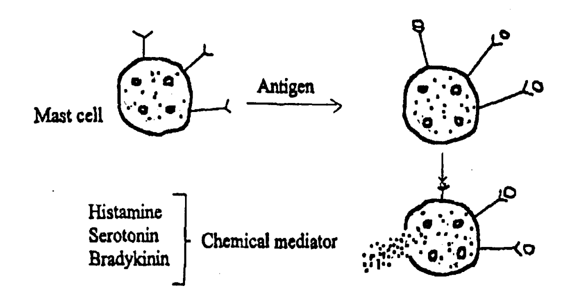
IgM pentamer

Immunoglobulin D:

- It is present as monomer and its heavy chain is δ chain.
- It is present m traces in serum 0.1-40 mg/dl.
- It is antibody -St insulin and food toxins.
- It can not activate complement.

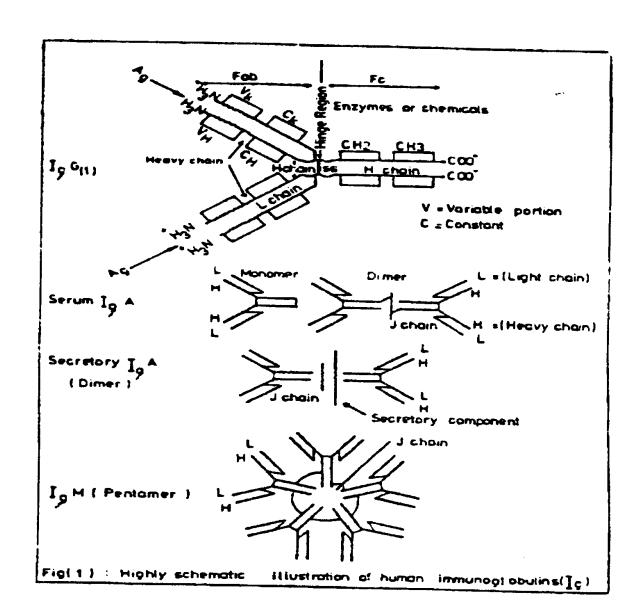
Immunoglobulin E:

- It is present m very low concentration in scrum 0.01-0.9 mg/dl.
- Its heavy chains are epsilon ε chains.
- It has the ability to bind firmly by Fc to basophils and mast cells.
- When the antigen combines with the cell-bound IgE a subsequent reactions occur and lead to destruction of cell membrane that leads to release of some chemical mediators.



These chemical mediators can cause allergy Histamine and Bradykinin cause vasodilatation of small blood vessels of skin leading to urticaria. Serotonin can cause vasoconstriction and bronchoconstriction leading to bronchial asthma.

- IgE is incrcascd in allergic and parasitic diseases.
- It can not activate complement.



Fig(1) : Highly schematic illustration of human immunoglobulins(Ig)

The complement:

— They are fractions of plasma proteins present in an inactive form and activated only by antigen-antibody reaction.

— They are present in α_1, α_2, β and γ globulins and named C1, C2, C3, C4, *C5*, C6, C7, C8 and C9.

— It is noticed that C1 consists of 3 subunits, $C1_q$, $C1_r$ and $C1_s$.

Activation:

— Complement can be activated by two systems : the classic pathway and the alternate pathway.

— Activation of the complement occurs by the removal of small polypeptide and the active form is indicated by a bar i.e. C_1.

Classic pathway:

It is activated by IgG_1, IgG_2, IgG_3 and IgM

Antigen-antibody reaction

↓

C1 ⟶ **$\overline{C1}$**

$C1_q$, $C1_r$ & $C1_s$ ↓

C42 ⟶ **$\overline{C42}$**

↓

C3 ⟶ **$\overline{C3}$**

↓

$\overline{C567}$ ⟵ **C567**

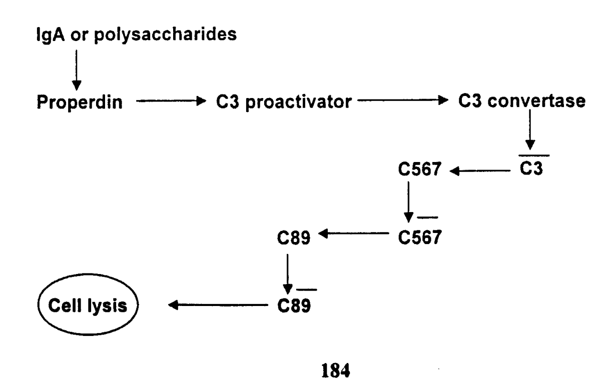
↓

Cell lysis ⟵ **$\overline{C89}$** ⟵ **C89**

This active form of *C5*, C6, C7, CS and C9, *C567* and C89 will create changes in the structure of the cell membrane leading to it lysis.

Alternative pathway:

It is activated by IgA and polysaccharides:



The active form of complement 8 and 9 (C89) is an enzyme that will load to cell lysis

Types of humoral immune response:

1. Anaphylactic type:

- This type of response is mediated by IgE.
- The antibody IgE is attached to basophil. or mast cells.
- When antigen combines with this IgE reactions occur and lead to destruction of cell membrane and release of some chemical mediators e.g. histamine, bradykinin and seratonin.
- These chemical mediators will lead to allergic manifestation as urticaria due to vasodilation of small blood vessels in the skin by the action of histamine and bradykinin. Also, lead to broncho and laryngospasrn due to the constrictor action of serotonin on smooth muscles.

2. Cytotoxic type:

In this type the antigen present on the cell membrane and when combine with the antibody it will lead to destruction of cell membrane e.g. red cells carry an antigen on their membrane which can combine with the antibody causing destruction of RBC and hemolysis. This will lead to hemolytic anaemia due to Rh incompatibility.

This type of immune response is mediated by IgG and IgM.

3. *Immune complex type:*

In this type the antigen is present in the blood and it combines with the antibody, then they circulate in the blood and may deposit in some tissues causing some diseases e.g. systemic lupus erythematosis. It is mediated by IgG and IgM.

1) Primary immune response:

Def.: It is the response which occurs when foreign body enter the body for the first time.

Characters:

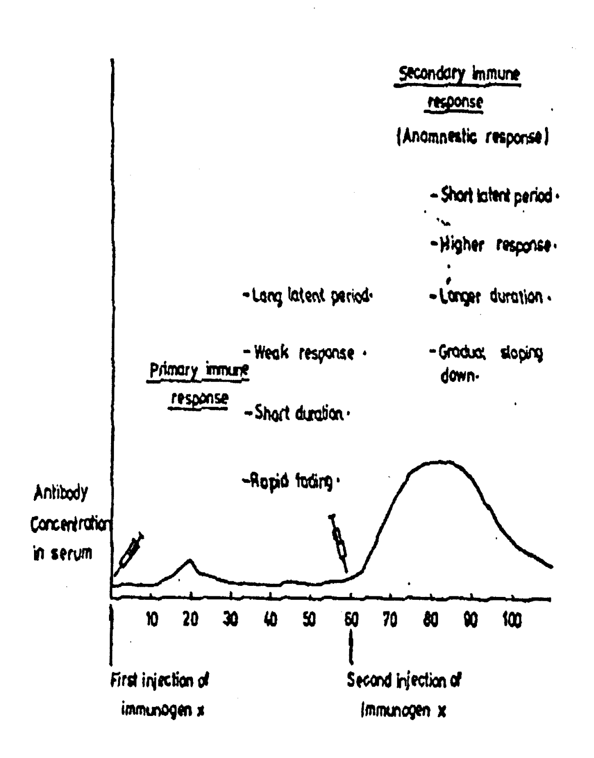
- It is of long latent period.
- It is a weak response.
- It is of short duration.
- It is of IgM type.
- Rapid fading.

(2) Secondary immune response:

Def.: It is the response which occur due to repeated entery of the foreign body in the body.

Characters:

- It is of short latent period.
- It is of strong response and long duration.
- It is of IgG type.



Immune response

ENZYMES

ENZYMES

- An enzyme is a protein that is synthesized in a living cell to speed up (accelerate) a biological reaction in the cell.
- All enzymes are protein in nature. So have the same properties of proteins as denaturation, priciptation, electrophoresis ... etc.
- Most enzymes belonged to simple proteins, some required non protein fractions which may loosly or firmly attached to them. This type is conjugated proteins.
- Enzymes are extracellular *e.g.* digestive enzymes or intracellular *e.g.* other body enzymes.
- Catalysts are substances that accelerate chemical reactions. They undergo chemical changes during a reaction but return to their original state at the end of the reaction. The protein catalysts are called enzymes.

Types of catalysts

1) Protein catalysts "enzymes".

2) Non protein catalysts *e.g.* H^+, HO^- and metals.

Difference between enzymes (biocatalysts) and inorganic catalysts are summarized in the following table:

Inorganic catalysts	Enzymes
1. Accelerate the reaction.	**1. Initiate the reaction.**
2. The reaction may occur in their absence but at a very low velocity.	**2. Many reactions do not occur in their absence.**
3. Their work is not specific i.e. accelerate different reactions.	**3. Their action is highly specific**
4..Unchanged by the reactions which they catalyze.	**4. Destroyed to an extent.**
5. Heat stable.	**5. Heat labile i.e. lose their activity at high temperature.**
6. Their action is irreversible	**6. Their action is reversible.**
7. Needed in bigger amounts	**7. Needed in traces.**
8..Have different chemical structure.	**8. All are proteins in nature.**

Cofactors are molecules needed for enzyme catalysed reactions. They are either:

1) Organic Cofactors that are called coenzymes and these are either covalently or non-covalently bound to the enzyme-
2) Inorganic Cofactors *e.g.* metal ions.

Nomenclature of Enzymes

Addition of the suffix "-ase" to the name of the first compound - the substrate- on which the enzyme was observed to act *e.g.*:

Substrate:	Enzyme:
Urea	Urease.
Arginine	Argininase.
Cellulose	Cellulase.

The suffix "-ase" may be added to the name of the chemical reaction carried out by the enzyme *e.g.*

Decarboxylase ⟶ Splites CO_2 from carboxylic acid

- However, a single compound could act as a substrate for a number of different enzymes and so undergoes various chemical changes.
- The names given to the enzymes often specified both the substrate and the reaction *e.g.*:
  - Pyruvic carboxylase.
  - Pyruvic dehydrogenase.
  - Pyruvic phosphokinase.

Chemical Nature of Enzymes

The enzymes are divided chemically into two groups:

(1) Simple proteins:

Consisting entirely of polypeptide chains, *e.g.* the hydrolytic enzymes pepsin trypsin, lysozyme and ribonuclease.

(2) Conjugated proteins:

Having some non protein component associated, whose presence is essential for enzymatic activity. These non-protein components are called cofactors.

The cofactor may be:

a) A metal ion.

a) An organic molecule called coenzyme.

Some enzymes require both.

- Cofactors are generally heat stable whereas most enzyme proteins loss activity on heating.
- The catalytically active enzyme cofactor complex is called holoenzyme.
- When the cofactor is removed the remaining protein which is catalytically inactive by itself, is called apoenzyme.

Holoenzyme = Apoenzyme + cofactor

- Enzymes requiring metal ions are sometimes called metalloenzymes.

Examples of some enzymes containing or requiring metal ions as cofactors:

Cofactor	Enzyme
Zinc (Zn^{2+})	**Alcohol dehydrogenase, carbonic anhydrase carboxypeptidase**
Magnesium (Mg^{2+})	**Phosphohydrolase, phosphotransferase**
Manganese (Mn^{2+})	**Arginase, phosphotransferase**
Iron (Fe^{2+} or Fe^{3+})	**Cytochrome peroxidase, catalase**
Copper (Cu^{2+})	**Tyrosinase, cytochrome oxidase**
Potassium (K^{+})	**Pyruvate kinase (also requiring Mg^{2+}**
Sodium (Na^{+})	**Plasma membrane ATPase (also requiring K^{+} and Mg^{2+}**

Metalloenzymes:

Enzymes contain metal ions tightly bound to them as:

Zn^{2+} ⟶ in carbonic anhydrase.

Fe^{2+} ⟶ in cytochrome catalase.

Cu^{2+} ⟶ in cytochrome oxidase.

Metal activated enzymes:

Enzymes that require metal ions for their activities

Mg^{+2} ⟶ phosphokinase.

Ca^{+2} ⟶ thrombokinase.

Cl^{-} ⟶ salivary amylase.

N.B. → 25% of enzymes are either metalloenzymes or metal activated enzymes.

General Properties of Enzymes

1) All enzymes are globular protein macromolecules with molecular weight 10,000 - 2,000,000.
2) The molecules of each enzyme have a characteristic conformation (3-dimensional shape) which is a property of all protein molecules and is the key to many of their biological functions.

3) Enzymes can be denatured: Like other proteins, the characteristic conformation of an enzyme molecule can be changed by extremes of temperature, pH; heavy metals, organic solvents and concentrated salt solutions.
4) Enzymes are functionally specific, this specificity is due to their conformation.
5) Enzymes catalyze only one or two reactions at the most.
6) Some are absolutely specific for only one chemical group (methyl group or amino group) or one particular type of chemical bond (peptide bond).

Types of specificity of enzymes:

a) Optical specificity: (Stereochemical specificity)

The enzymes will act according to the spatial configuration of certain groups in their substrates.

Maltase: → catalyzes the hydrolysis of α- but not β- glycosidic linkage.

Amylase: → attacks the α-glycosidic linkage of the starch.

Cellulase: → attacks the β- glycosidic linkage.

D- and L-amino acid dehydrogenases: → act on D- and L-amino acids (catalyze oxidative deamination of amino acids).

α-amino acid **Oxidativa deamination** → α-keto acid
↘ **NH_3**

b) Group specificity:

e.g. pepsin and trypsin act on peptide bonds.

Pepsin: → acts on peptide bond between amino group of aromatic amino acid and carboxyl group of other amino acid.

Chymotrypsin: → acts on peptide bond between **-COOH** of aromatic amino acid and **$-HN_2$** of other amino acid.

Carboxypeptidase: → splits off amino acid at **-COOH** terminal. end of polypeptide chains.

Aminopeptidase: → splits off amino acid at **$-NH_2$** terminal end of polypeptide chain.

c) Relative specificity:

The enzyme may have ability to attack various substrates but at different rates *e.g.* pancreatic esterase hydrolyzes aliphatic esters (FA + alcohol) rapidly but cholesterol ester slowly (cholesterol + FA).

d) Absolute specificity:

Enzymes act on only one substrate *e.g.* urease acts on urea, arginase acts on arginine. So, a large number of different enzymes are required for the many complex biochemical reactions.

7) Enzyme activity depends on temperature and pH factors:
Optimum = The most favourable condition under which enzymes are most active.

8) Enzymes are not used up or permenantly changed by the chemical reactions they catalyze. This does not mean that enzyme molecules lost activity. All biomolecules in the body are sooner or later broken down and replaced⟶ turnover.

9) An enzyme may exist In two or more different molecular forms.

- Different forms of the same enzyme are called isoenzymes or isozymes.
- Isoenzymes may vary in the rate at which they catalyze the same chemical reaction *e.g.* S- isoenzymes of lactic dehydrogenase (an enzyme that catalyzes an energy - producing reaction in muscles).

- The isoenzyme varient with the most rapid rate of catalysis is found in muscles that are continuously active.
- Isoenzymes thus represent a biological adaptation to the functional requirements of different types of tissue in the body.
- The molecule of lactic dehydrogenase is composed of 4 subunits of approximately the same size but of two types H and M and them combinations of the two are possible *i.e.*, H_4 (LD_1), H_3M (LD_2), H_2M_2 (LD_3), HM_3 (LD_4) and M_4 (LD_5).

10) Some enzymes are produced in the form of an inactive precursor
    - a proenzyme, or zymogen. These enzymes are mainly proteolytic enzymes (proteo: protein lysis = dissolving). Such a mechanism evolved safety precuation.
    - If an active proteolytic enzyme was synthesized and turned loose in a cell, it could destroy the structural and functional proteins of the cell *e.g.* proteolytic digestive enzymes.
11) Many enzymes require a cofactor in order to be functional.

    Apoenzyme + cofactor = Holoenzyme.

    A-Non-protein organic compound.

    B- Metal ions.

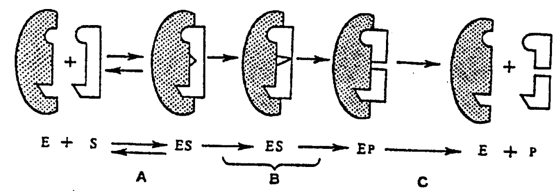
If the cofactor is firmly attached → prosthetic group.

- A coenzyme is not necessarily specific for only one enzyme. groups of apoenzyme often operate with the same coenzyme.
- Many of the coenzymes in the body are derivatives of vitamin B complex.

Mechanism of action of enzymes

The loops and folds of the polypeptide chains of enzyme molecules form cervices and pockets by which they recognize and bind specific smaller molecules that fit into them.

* Active site: It is the binding site on the enzyme molecule where the catalytic action takes place.
* The interaction between enzyme and substrate will occur by bonds that are mainly hydrogen bonds, relatively weak non-covalent, easily broken and reformed.



(E—enzyme; S—substrate, P—product)

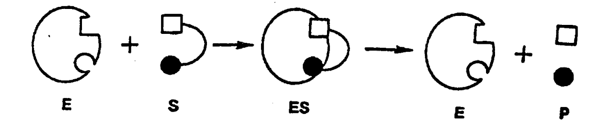
A: Substrate molecule fits into active site on surface of enzyme molecule.

B: An enzyme-substrate complex is formed and a chemical reaction is catalyzed in which substrate is changed.

C: The end product(s) of the reaction dissociate from the unchanged enzyme molecule.

— A substrate must have a matching shape to fit into the active catalytic site:—

1) Lock and key (Template) model:



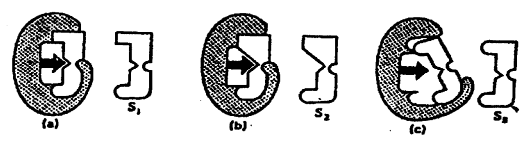
Representation of formation of an ES complex according to template hypothesis.

E: enzyme, S: substrate, P: Product(s), ES: enzyme substrate complex

The active catalytic site of enzyme by itself is pre-shaped to fit the substrate i.e. is complementary in shape to that of substrate.

2) Induced fit model:

The catalytic site of some enzymes may not be rigid i.e.. the shape of active catalytic site is modified by the binding substrate. It has a shape complementary to that of the substrate only after the substrate is bound to the enzyme.

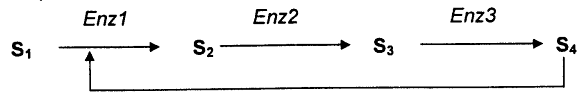


Induced fit model for enzyme-substrate interaction.

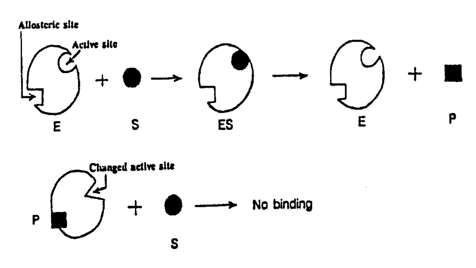
a) **best fit of catalytic groups after the contact of enzyme with substrate (true substrate).**
b) **the accommodation of catalytic groups enables the enzyme to act on the substrate, but at a slower rate.**
c) **Mismatch of catalytic groups prevents the conversion of substrate.**

Regulatory (allosteric) enzymes:

If the reaction passes in a manner like the following sequence:



- The end product of the pathway may inhibit the enzyme that initiates this pathway. *i.e.* the end product regulates its own synthesis.
- This is called feedback inhibition and Enz1 is a regulatory or key enzyme.
- The regulatory enzyme has an active site for binding to the substrate (as all enzymes) and an additional site for binding to the product called the allosteric site.
- The substance that binds to the allosteric site is called modifier or effector.
- When the effector binds to the allosteric site of the enzyme it causes a confermational changes in enzyme structure and hence changes the shape of the active site.
- So the enzyme is unstable to recognize its substrate *i.e.* enzyme is inhibited.



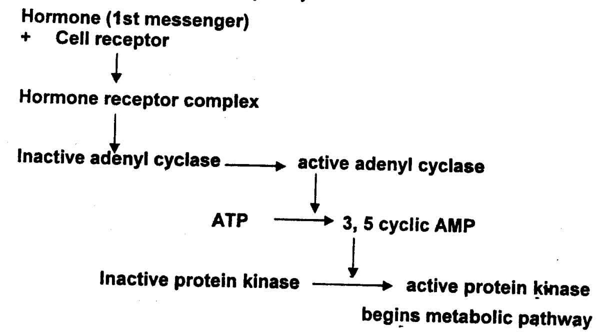
(E: enzyme, S: substrate, P: product).

Enzyme induction:

- The enzyme synthesis (as any protein) is genetically controlled.
- If the cell is always exposed to a specific substance (inducer), the gene will initiate the synthesis of much enzyme molcules specific for this substance. This enzyme is called Induced enzyme.
- If enzyme concentration in the cell is constend and independent on inducers, the enzyme is called constitutive enzyme.

Enzyme hormone interaction:

Hormones are chemical messengers sent by certain cells to regulate the activity of other cells (target cells). The effect of hormones are mediated by enzymes.



Differences between hormones and enzymes

	Enzymes	Hormones
Structure	Protein	Some are proteins (insulin)
Site of action	At same site they are formed	Away from the site they are formed
Mechanism of action	On specific subst.	Different mechanisms of action
Site of secretion	No specific sites.	By specific endocrine glands

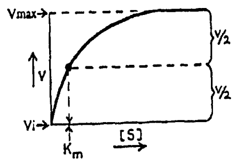
Factors affected enzyme activity (enzyme kinetics)

Various factors influence the velocity of enzyme reactions. Among these are:

(1) **Conenetration of the substrate:**

- The principles of chemical-reaction kinetics apply to enzyme-catalyzed reaction, but they also show a distinctive feature not usually observed in non-enzymatic reactions saturation with substrate.

non-enzymatic reactions saturation with substrate.



Effect of substrate concentration on the velocity of an enzyme-catalyzed reaction.

V: velocity S: substrate

Vi : initial velocity

Vmax: velocity at its maximal value

Km: concentration of substrate that produces 1/2 of maximal velocity (1/2 Vmax)

<u>Michaelis-Menten expression:</u>

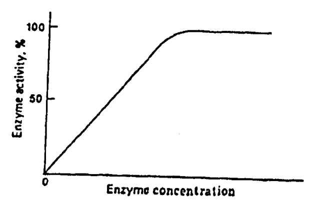
$$Vi = \frac{Vmax\ (S)}{Km\ (S)}$$

- If the rate of the reaction is measured in the presence of different concentrations of substrate but with a constant amount of enzyme, the result usually have the form shown above.
- At low concentration of substrate the rate is dependent on the concnetration and increases as this is raised, but eventually approaches a maximum value where it is almost independent of the concentration.
- The substrate is now said to be in excess and the enzyme is said to be saturated with its substrate.

- All enzymes show the saturation effect, but they vary widely with respect to the substrate concentration required to produce it.

(2) Enzyme concentration:

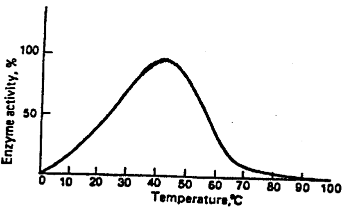
The velocity of an enzyme-catalyzed reaction is directly proportional to the enzyme concentration until a point where further increase in enzyme concentration will have no effect.



(3) Temperature:

The rate of most chemical reactions is approximately doubled or trebled for each 10°C rise in temperature and this is true for enzymic reaction up to about 40-50°C, but thermal denaturation of the enzyme occurs leading to reduced activity and the rate falls and most enzymes show complete loss of activity if kept at 60 – 70°C.

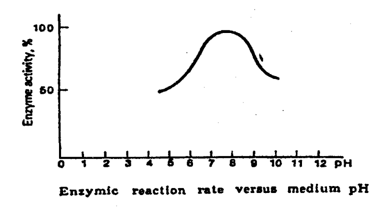
For most enzymes, optimal temperature approximate those of the environment of the cell



Enzymic reaction rate versus temperature

(4) pH hydrogen ion concentration:

Most enzymes have a characteristic pH at which their activity is maximal, above or below this pH the activity declines.



Enzymic reaction rate versus medium pH

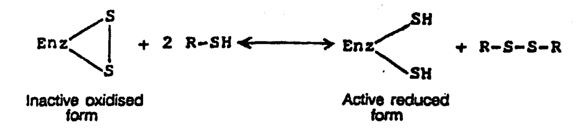
The shape of pH-activity curve is determined by the following factors:

1. Enzyme denaturation at extremely high or low pH values.
2. Effects on the charged state of the substrate or enzyme. (the effect of a change in pH on an enzyme molecule is to alter the state or degree of ionization of acidic and basic groups).

(5) Oxidation:

The sulfhydryl groups (-SH) of many enzymes especially the oxido-reductases (dehydrogenases) are essential for enzymatic activity. Oxidation of these -SH groups, forming disulfide linkages (S-S) brought about by many oxidizing agents including O_2 of air results in loss of activity.

Frequently this also may cause a conformational change in the enzyme. Full activity may often be restored by reduced -SH compounds such as glutathione or cysteine (R-SH). These reduce the enzyme S-S to -SH by disulfide exchange.

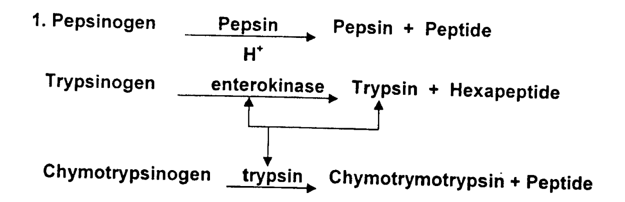


(6) Radiation:

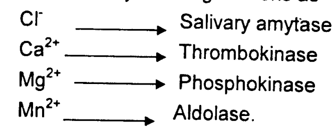
Enzymes are highly sensitive to short wavelength (high energy) radiation such as ultraviolet light, X-rays, β or γ-rays. This is in part due to oxidation of the enzyme by peroxidases formed by high-energy radiation. In the intact cell, loss of enzyme activity upon irradiation may also be due to indirect effects on the DNA of genes.

(7) Activators:

These are substances which specifically increase the activity of a complete enzyme.



2. Glutathione and cysteine are activators for enzymes with -SH groups in their molecules.
3. Activators may be inorganic ions as



(8) Inhibitors:

The rate of enzyme reaction may be greatly reduced or the reaction stopped by the addition of some reagent which may be of no action on some other enzymes. There are thus specific inhibitors for enzymes.

Types of enzyme inhibitors:

I- *Competitive or substrate analog inhibitors (Reversible inhibitor)*

- Each enzyme has an active site (Catalytic site) where the enzyme binds the substrate and the catalytic function takes place.
- The competitive inhibitor resembles more or less chemically the substrate. So the inhibitor will compete with the substrate at the active site.
- The inhibitor may fit into active site of the enzyme.
- Once inhibitor bound to the enzyme, enzyme can not convert inhibitor to product because some detail of its structure is wrong.

$$E + S \longleftrightarrow ES \longleftrightarrow E + P$$

$$E + I \longleftrightarrow EI \quad \text{(no further reaction)}$$

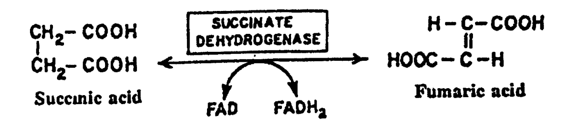
(E: enzyme, S: substrate, I: inhibitor, P: product).

The inhibitor depends on:

a- Affinity of the inhibitor for enzyme. If this affinity is greater, the more EI formation and leaves less enzyme for substrate formation.

b- Relative amounts of substrate and inhibitor present since they are competing with each other for the same active site.

- Competitive inhibitors are inhibitors whose action can be reversed by increasing amounts of substrate.

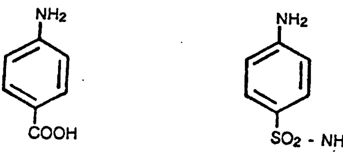


COOH
|
CH_2
|
COOH

Malonic acid

Malonic acid is competitive inhibitor for succinic acid dehyrogenase as its structure resembles that of succinic acid.

- Dicumarol that has similar structure of vit. K inhibits prothrmbin activation.
- Sulphonamides compete with para-aminobenzoic acid which is used by micro-organism for synthesis of folic acid that is essential for purine and thymine bases.
- So sulphonamides arrest the growth of these micro-organisms.



Para-aminobenzoic acid

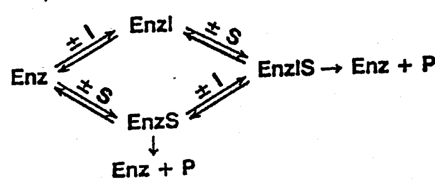
Sulfonamide

II- Non Competitive inhibitors

a) Reversible inhibitors:

- In this type, inhibitor (I) has no structural resemblance to the substrate (S) and may be assumed to bind to enzyme (E) at a different region.
- Since I and S may combine at different sites, so formations of EI and EIS complexes are possible.
- EIS complex may break down to give rise to products at slower rate than does ES. So the reaction will occur at slower rate but not stopped.
- So, I and S can bind simultaneously to E, *i.e.* their binding sites do not overlap.

- The inhibitors produce conformational changes in E → change in its shape but this is not significant.



Enz.: enzyme, S: substrate, I: inhibitor, P: product

Examples:

Oxidation of-SH group. It will regain activity after reduction.

b) Irreversible non-competitive inhibitors:

The I does not resemble the S in the chemical structure but it is a reactive molecule which combines with some important groups in E, *i.e.*, I reacts covalently with E.

There is no relation between I and S. So at any concentration of I, it will depress the enzymatic reaction and increasing amount of S will not regain the enzymatic activity.

Examples:

1- Cyanide inhibits iron containing enzymes *e.g.* cytochrome oxidase, catalase and peroxidase.
2- Fluoride inhibits enzymes requiring Mg^{2+} *e.g.* hexokinase.

III- Allosteric inhibitors (feed-back inhibitors):

This type of inhibition means inhibition of the activity of enzyme in a biosynthetic pathway by an end product of that pathway.

This type of inhibition is reversible as when the concentration of the product drops, the enzyme becomes active again and the product will be formed.

Examples:

- Glycogen inhibits glycogen synthase.
- Cholesterol inhibits HMG-COA reductase.
- G-6-p inhibits Hexokinase.

(9) Effect of product concentration:

Increased product concentration inhibit enzyme activity due to

1- Change in Ph of the medium.
2- Similarity in structure to substrate and may compete with it to enzyme

(10) Effect of coenzyme concentration:

Like substrate concentration as it combines like it with enzyme.

(11) Effect of time:

The time is essential to define the various conditions affecting the rate of enzymatic activity, optimum pH and optimum temperature should be expressed relative to the time of the reaction

CLASSIFICATION OF ENZYMES

Enzymes are calssified on the basis of the reaction catalysed into

1) **Oxidoreductases:** Involved in oxidation and reduction reactions.
2) **Transferases:** Transfer functional groups.
3) **Hydrolases:** Catalyse hydrolysis of substrates.
4) **Lyases:** Cleave substrates without addition of water.
5) **Isomerases:** Catalyse changes within one molecule.
6) **Ligases:** Join two molecules.

I-OXIDO-REDUCTASE

They are classified into 5 subgroups

1) **Oxidases**

Enzymes that catalyze removal of hydrogen from substrates but use oxygen as hydrogen acceptor and form water as a reaction product, (except uricase and monoamine oxidase which produce H_2O_2 instead of water).

They are conjugated proteins containing copper.

Examples:

*** Cytocnrome oxidase:**

It is the terminal component of the respiratory chain. It is pointed with CO, H_2S and cyanide.

It contains 2 atoms of copper and 2 atoms of iron and the latter oscillate between Fe^{2+} and Fe^{3+} in oxidation reduction

*** Tyrosinase** : Tyrosine $\longrightarrow$ Melanin

*** Uricase** : Uric acid $\longrightarrow$ Allantion

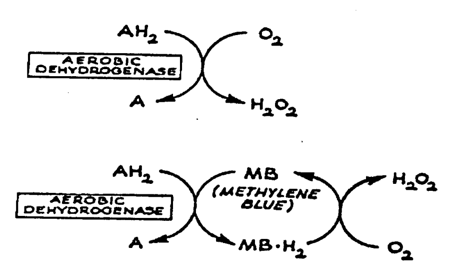
* **Monoamine oxidase**: in catabolism of epinephrine and norepinephrine.

AH_2 (RED) → A (OX); ½ O_2 → H_2O; OXIDASE

Oxidation of a metabolite catalyzed by an oxidase.

2) Aerobic dehyrogenases:

- Enzymes catalyzing removal of H_2 from substrate but which as distinct from oxidases.
- They can use either O_2 or artificial substances as methylene blue as a hydrogen acceptor.
- These dehydrogenases are flavoproteins. H_2O_2 is formed as a reaction product.



Oxidation of a metabolite catalyzed by an aerobic dehydrogenase

- Many of these enzymes contain in addition a metal which is essential for the functioning enzyme, so they are called metalloflavoproteins.

Examples:

*** L-amino acid dehydrogenases** [FMN-linked enzymes]

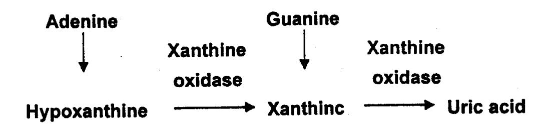
Used in oxidative deamination of naturally occurring L-amino acids.

COOH | H_2N-CH | R —(FMN → FMNH2)→ COOH | HN=C | R —(H2O → NH3)→ COOH | C = O | R

L-amino acid — Imino acid — Keto acid

*** Xanthine oxidase (dehydrogenase)** [FAD-liked enzymes]

They contain iron and molybdenum in addition. They catalyze conversion of bases to uric acid.



3) Anaerobic dehydrogenases:

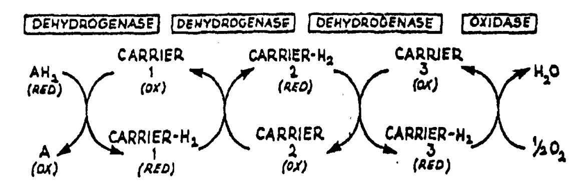
Enzymes catalyzing removal of H_2 as hydrogen açceptor. There a substrate but not to use O_2 as hydrogen acceptor. There are large numbers of these enzymes. They perform two main functions:

a- Transfer of H_2 from one substrate to another in a coupled oxidation-reduction reaction not involving respiratory chain.

AH2 (RED) — CARRIER (OX) — BH2 (RED)
A (OX) — CARRIER-H2 (RED) — B (OX)
DEHYDROGENASE SPECIFIC FOR A — DEHYDROGENASE SPECIFIC FOR B

Oxidation of a metabolite catalyzed by anaerobic dehydrogenases, not involving a respiratory chain

b- As components in the respiratory chain of electron transport from substrate to oxygen.



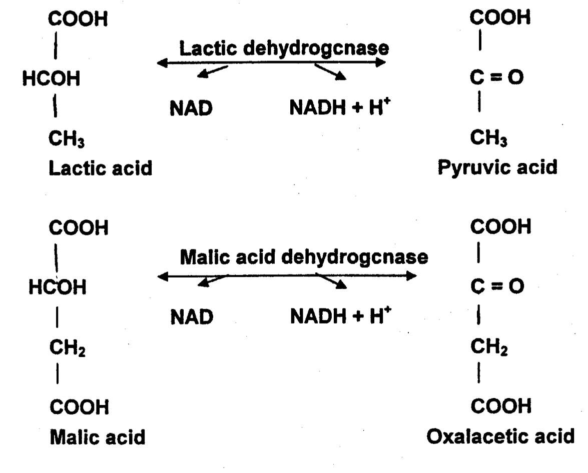
Oxidation of a metabolite by anerobic dehydrogenases utilizing several components of a respiratory chain

Examples:

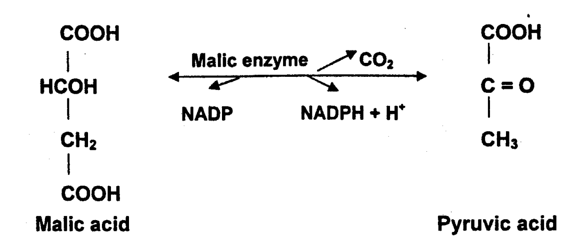
1- Dehydrogenases dependent on Nicotinamide coenzymes

* NAD- linked dehydrogenase

Catalyze oxido-reduction reactions in the oxidative pathways of metabolism as glycolysis, citric acid cycle and in respiratory chain



* **NADP -linked dehydrogenases** in reductive biosynthesis as in extramitochondrial pathway of fatty acid synthesis and in steroid synthesis. Also they are used as coenzymes for dehydrogenases of hexose monophosphate shunt.



ii- Dehydrogenases dependent On Riboflavin prosthetic groups (FMN, FAD)

Most of them are concerned with electron transport in the respiratory chain.

$$\begin{array}{c} COOH \\ | \\ CH_2 \\ | \\ CH_2 \\ | \\ COOH \end{array} \xrightarrow[FAD \rightarrow FADH_2]{\text{Succinic acid dehydrogenases}} \begin{array}{c} COOH \\ | \\ CH \\ | \\ CH \\ | \\ COOH \end{array}$$

Succinic acid **Fumaric acid**

iii- Cytochromes

Except for cytochrome oxidase, cytochromes are classified as anaerobic dehydrogenases. In respiratory chain, they are involved as carries of electrons from flavoprotein on one hand and to cytochrome oxidase on the other hand They are iron-containing haemoproteins ($Fe^{2+} \rightarrow Fe^{3+}$) during oxidation reduction process.

They are cytochromes b, c1, c2, a and a3 (cytochrome oxidase).

4- Hydroperoxidases

Enzymes utilizing H_2O_2 as substrate:-

- **a- Peroxidase:** present in milk and leukocytes

$$\underset{(red)}{H_2O_2 + AH_2} \xrightarrow{\text{Peroxidase}} \underset{(oxd)}{2\ H_2O + A}$$

- **b- Catalase:** present in blood and liver.

Its function is destruction of H_2O_2 formed by the action of aerobic dehydrogenases.

$$2\ H_2O_2 \xrightarrow{\text{Catalase}} 2\ H_2O + O_2$$

5- Oxygenases

Enzymes catalyze the direct transfer and incorporation of O_2 into a substrate molecule:–

– **a) Dioxygenase**

Catalyzes incorporation of both atoms of O_2 into the substrate.

$$A + O_2 \longrightarrow AO_2$$

Example:

* Tryptophan pyrrolase

$CH_2-CH(NH_2)-COOH$ $\xrightarrow[\text{Tryptophan pyrrolase}]{[O_2] \quad Fe}$ $C(=O)-CH_2-CH(NH_2)-COOH$, $N(H)-CHO$

L-TRYPTOPHAN → *N-FORMYLKYNURENINE*

- Homogentisic acid dioxygenase.
- 3-hydroxy anthranilic acid dioxygenase.

– **b) Monoxygenase (Hydroxylase)**

Catalyze incorporation of only one atom of O_2 into the substrate. The other atom of O_2 is reduced to H_2O

$$A\text{-}H + O_2 + Z.H_2 \longrightarrow A.OH + H_2O + Z$$

($Z.H_2$ ↓ CO. substrate or electron doner)

N.B. Many enzymes used in steroid synthesis or transformation are mono-oxygenase using NADPH as a CO- substrate.

II- TRANSFERASES

Enzymes catalyzing a transfer of a group (other than hydrogen) between a pair of substrates.

$$SG + S' \longleftrightarrow S + S'G$$

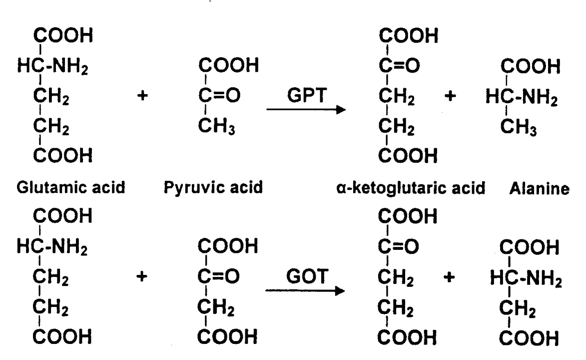
1) Transaminases (Aminotransferases):

These transfer amino group (-NH2) from an amino acid to α-keto acid thus producing new amino acid.

The coenzyme of transaminases contains vit. B_6 as pyridoxal phosphate.

α-amino acid → α-Keto acid (B6 Transaminase)
α-Keto acid ← α-amino acid

α-Amino acid + α-Keto acid ⇌ (Transaminase, B6) α-Keto acid + α-Amino acid



GPT = gluamic pyruvic transaminase.
GOT = Glutamic oxaloacetic transaminase.

2) Transmethylases (Methyl transferase):

– These enzymes transfer methyl group ($-CH_3$) from certain compounds which act as methyl donors to other methyl acceptor. ***Examples*** of methyl donors include betaine, choline and methionine. Methionine is an important methyl donor. It is first activated and its methyl group becomes labile.

Methionine + ATP → S-adenosyl methionine + 3 H_3PO_4

– S-adenosyl methionine is active methionine. Its methyl group can be transferred to a suitable acceptor through a specific transmethylase enzyme.

Examples: of transmethylation reactions:

$$NH_2\text{-}CH_2\text{-}COOH \xrightarrow[\text{Transmethylase}]{+3\ CH_3} (CH_3)_3N^+\text{-}CH_2\text{-}COOH$$

Glycine → **Betaine**

$$OH\text{-}CH_2\text{-}CH_2\text{-}NH_2 \xrightarrow[\text{Transmethylase}]{+3\ CH_3} HOCH_2\text{-}CH_2\text{-}N^+(CH_3)_3$$

Ethanolamine → **Choline**

$$\text{noradrenalin } (CHOH\text{-}CH_2NH_2) \xrightarrow[\text{Transmethylase}]{+\ CH_3} \text{adrenalin } (CHOH\text{-}CH_2NH\text{-}CH_3)$$

noradrenalin → adrenalin

$$NH_2\text{-}C(=NH)\text{-}NH\text{-}CH_2\text{-}COOH \xrightarrow[\text{Transmethylase}]{+\ CH_3} NH_2\text{-}C(=NH)\text{-}N(CH_3)\text{-}CH_2\text{-}COOH$$

guanidinoacetic acid → creatine

3) Transacylases (Acyl Transferases):

These enzymes transfer acyl group. They need coenzyme A which acts as a carrier for the acyl group.

a- <u>Transacetylation</u>: It is an important process in the body. Active acetate (acetyl CoA = $CH_3 - CO \sim SCOA$) is formed from oxidative decarboxylation of pyruvic acid or from oxidation of a fatty acid. This active acetate is carried by CoA and thus called (acetyl COA).

Acetyl CoA is transferred to oxalacetic acid to from citric acid and then oxidized to CO_2 and H_2O through citric acid cycle.

<u>Examples of transacetylation reactions:</u>

1) Formation of citic acid from oxalacetic and acetyl CoA.
2) Formation of acetic acid and fatty acids.
3) Biosynthesis of cholesterol in the body from acetyl CoA.
4) Formation of acetyl choline.

Choline + Acetyl CoA $\xrightarrow{\nearrow \text{CoASH}}$ **acetyl choline**

b- <u>Transacylation</u>: Transfer of acyl group attached to CoA other than acetyl CoA.

Examples:

Succinyl CoA + glycine $\xrightarrow{\nearrow CO_2 \ \nearrow \text{CoASH}}$ **6-amino levulenic acid**

This is the first step in the formation of prophyrin ring for haemoglobin synthesis.

Benzoyl CoA + glycine $\xrightarrow{\nearrow \text{CoASH}}$ **Hippuric acid**

4) Transphosphatases

a- Kinases: Transfer phosphate group from an organic compound to substrate or from substrate to ADP to from ATP:–

Examples:

$$\text{Glucose} \xrightarrow[\text{Hexokinase}]{\text{ATP} \searrow \; Mg^{2+} \; \nearrow \text{ADP}} \text{Glucose-6-phosphate}$$

1,3-Disphosphoglycerate ⇌ (PHOSPHOGLYCERATE KINASE; ATP, ADP) Glyceraldehyde 3-phosphate

b- Mutases: Transfer group from one carbon to another in the same molecule.

$$\text{Glucose-6-phosphate} \xleftrightarrow{\textit{Pbosphoglucornutase}} \text{Glucose-l -phosphate}$$

5) Transglycosidases:

A number of enzymes which add phosphoric acid (or remove it) and produce changes in the substrate (Phosphorylases)

Examples

$$\underset{(n)}{\text{Glycogen}} + H_3PO_4 \xrightarrow{\textit{Glycogan phosphorylasc}} \text{G-1-P} + \underset{(n-1)}{\text{Glycogen}}$$

$$\text{G-1-P} + \text{UTP} \xrightarrow{\text{UDP} \searrow \; \textit{Pyrophosphorylase}} \text{UDP-G} + \text{PPi}$$

III-HYDROLASES

These are enzymes that add water to the substrate to decompose it They are subdivided into

1 - Glycosidases

2- Esterases

3- Peptidases

4- Amidases

1) Glycosidases:

They split the higher carbohydrates into simpler ones.

a) Polysaccharidases:

* **Amylases** : act on α-1,4-glucosidic linkages in starch and glycogen molecules converting them into dextrins and maltose. Salivary amylase starts the digestion of starch and glycogen in the mouth and shortly after swallowing. It acts at pH of 6.8, so It is inhibited by gastric acidity.

  Pancreatic amylase completes the digestion of starch and glycogen in the small intestine. It has a complete action because it acts at its optimum pH (7.5-8) in the small intestine.

  Both amylases are activated by chloride ions.

* **Cellulase:** attacks β-1,4-glycosidic linkages of cellulose. it is not present in human intestine, so cellulose is undigestable in humans. Bacteria present in herbeverous animal intestine produce this enzyme and help these animals to benefit from cellulose by converting it into cellobiose.

* **Mucopolysaccharidase:** *e.g.* Hyaluronidase enzyme that is present in some insects and causes hydrolysis of hyaluronic acid in subcutaneous tissue.

b) disaccharidase:

* **Maltase:** acts on α-1,4-glucosidic linkage of maltose producing 2-α-glucose units.
* **Lactase:** acts on β-1,4-galactosidic linkage of lactose producing α-glucose and β-galactose.
* **Sucrase = Invertase :** acts on β-2,1-fructosidic linkage of sucrose producing α-glucose and β-fructose.

The 3 enzymes are intestinal and act at pH 7.5-8.

2) Esterases :

They attack ester linkages **"$R-\overset{O}{\overset{\|}{C}}-OR$"**

a) Lipases:

They split fatty acids from lipids.

* **Pancreatic lipase:** Splits 2 fatty acids from α- and γ-positions of triacylglycerols converting them into β-monoacylglycerols. It acts at pH 7.5-8 and is activated by bile salts.
* **Cholesterol esterase:** it is a pancreatic enzyme that splits the fatty acid from cholesterol esters producing free cholesterol. It acts at pH 7.5-8.
* **Lecithinase:** Splits a fatty acid from β-position of lecithine producing lysolecithine

b) Phosphatases:

They hydrolyse phosphoric acid esters in different tissues.

* **Acid phosphatase:**
* **Nucleotidase:** Splits phosphate group from nonucleotides converting them into mononucleosides.

c) Sulphatases:

They hydrolyse sulphoric acid esters in different tissues.

3) Peptidases (Proteolytic enzymes)

They split the peptide linkages of protein "- C(=O)- NH-"

a) Endopeptidases:

They split peptide linkages inside the peptide chain.

*** Pepsin:**

— Produced from the chief cells of the stomach as pepsinogen (zymogen) and activated after removal of a 44-amino acid peptide by HCl or another molecule of pepsin.

— It acts at pH 1-2 (*in stomach*). It acts at peptide linkages formed by amino groups of aromatic and dicarboxylic amino acids. It converts proteins into proteoses and peptones.

*** Cathepsin:**

— It is a gasteric enzyme (as pepsin). It completes the action of pepsin but at pH 3.5. it converts proteins into proteoses and peptones.

*** Rennin (chymosin):**

— It cleaves a peptide from caseinogen converting it into soluble casein, the latter is precipitated as Ca^{+} casienate that is the milk clot.

So rennin is called *milk clotting enzyme*.

— It acts at pH 3.5-4.5. So it is inactive in adult stomach, it acts only in infants.

*** Trypsin:**

— A pancreatic endopeptidase that is secreated as zymogen; trypsinogen. Its activation occurs via cleavage of a hexapeptide by enterokinase (another endopeptidase formed in the small intestine), or by another molecule of trypsin.

— Trypsin acts at pH 7.5-8 it attacks peptide bonds formed by carboxylic groups of basic amino acids convening proteins. proteoses and peptones into polypeptides.

*** Chymotrypsin:**

Another pancreatic endopeptidase that is secreted as chymotrypsinogen and its activation occurs by the effect of trypsin enzyme. Its optimum pH is 7.5-8. It hydrolyses the peptide bonds formed by carboxylic groups of neutral and aromatic amino acids. Chymotrypsin converts proteoses and peptones into polypeptides.

*** Elastase:**

Formed also by the pancreas. it is secreted as proenzyme (proelastase) and activated by trypsin that removes the inhibitory peptide.

It is specific for peptide bonds formed of small amino acids (glycine. hydroxyproline .. etc). It converts proteoses and peptones into polypeptides. it acts at pH 7.5-8.

*** Eneterokinase:**

An intestinal endopeptidase that attacks trypsinogen to remove the inhibitory hexapeptide and converts it into trypsin. Its optimum pH is 7.5-8.

b) <u>Exopeptidases:</u>

They split peptide linkages only at the end of the molecule.

***Carboxypeptidase:**

It is present in pancreatic juice. It is secreated as procarboxy peptidase and activated by trypsin. It attacks the carboxyl end of polypeptide liberating a free amino acid and acts at pH 7.5.

*** Amino peptidase:**

It is present in intestinal juice, contains Mg^{2+} and acts at ph 7.5. It attacks the peptide bond at the amino end of polypeptides producing a free amino acid.

*** Dipeptidase:**

It is present in intestinal juice, it attacks dipeptides to liberate two free amino acids, its optimum pH is 7.5.

4) **Amidases**

These hydrolyze C-N linkage other than peptide linkage.

*** Glutaminase:**

$$\underset{\text{L-Glutamine}}{\begin{array}{c} O \\ \| \\ C-NH_2 \\ | \\ CH_2 \\ | \\ CH_2 \\ | \\ H-C-NH_2 \\ | \\ COOH \end{array}} \xrightarrow[\text{GLUTAMINASE}]{H_2O \quad NH_3} \underset{\text{L-Glutamic acid}}{\begin{array}{c} O \\ \| \\ C-OH \\ | \\ CH_2 \\ | \\ CH_2 \\ | \\ H-C-NH_2 \\ | \\ COOH \end{array}}$$

*** Arginase** Arginine $\xrightarrow[+ H_2O]{\text{Arginase}}$ Urea + ornithine

*** Urease** Urea $\xrightarrow[+ H_2O]{\text{Urease}}$ CO_2 + $2NH_2$

*** Hippuricase** Hippuric $\xrightarrow[+ H_2O]{\text{Hippuricase}}$ benzoic acid + glycine

IV-LYASES

These enzymes catalyze removal of groups from substrates by mechanisms other than hydrolysis leaving double bonds.

$$\begin{array}{cc} X & Y \\ | & | \\ -C & -C- \end{array} \longleftrightarrow -C=C- + X-Y$$

Examples:

1) Aldolase:

Fructose 1,6 diphosphate $\xrightleftharpoons{\text{Aldolase}}$ Glyceraldehyde 3-P + Dihydroxyacetone-P

$$\begin{matrix} HO-CH-COOH \\ | \\ CH_2-COOH \end{matrix} \xrightleftharpoons{\text{Fumarase}} \begin{matrix} H-C-COOH \\ \| \\ HOOC-C-H \end{matrix} + H_2O$$

Malic acid → Fumaric acid

3) Carbonic anhydase:

$$H_2CO_3 \xrightleftharpoons{\text{Carbonic anhydrase}} H_2O + CO_2$$

V- ISOMERASES

These catalyse interconversion between isomers.

Isomerases:

$$\begin{matrix} CH_2\text{-O-P} \\ | \\ C=O \\ | \\ CH_2\text{-OH} \end{matrix} \xrightarrow{\text{Phosphotriose isomerase}} \begin{matrix} H\text{-}C=O \\ | \\ H\text{-}C\text{-OH} \\ | \\ CH_2\text{-O-P} \end{matrix}$$

Dihydroxy acetone-P → **Glyceraldehyde 3-P**

Mutases:

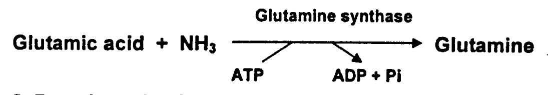
$$\text{Glucose} - 1 - \text{P} \xrightarrow{\text{Phosphoglucomutase}} \text{Glucose} - 6 - \text{P}$$

VI- LIGASES

These enzyme catalyze the linking of two molecules with breaking of P-P bond of ATP. They are called synthases.

Examples:

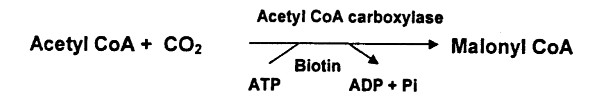
1- Glutamine synthase:



2. Pyruvic carboxlases:

$$\text{Pyruvic acid} + CO_2 \xrightarrow[\text{ATP} \quad \text{Biotin} \quad \text{ADP + Pi}]{\text{Pyruvate carboxylase}} \text{Oxalacetic acid}$$

3. Acetyl CoA carboxylase:



- Extracts of intestinal parasites *e.g.* ascaris contain substances which inhibit pepsin and trypsin. These substances are called antienzyme. For this reason the parasite worm is not digested in the intestine. (The inhibition occurs only around the parasite).
- Trypsin inhibitors are also found in raw egg white.

Autolysis:

Definition:

- It is the breakdown of cell proteins by intracellular enzymes known as cathepsins.
- These enzymes present in all tissues especially in the liver, spleen, kidney and stomach.
- In the living body these enzymes are usually inactive since their optimum pH is slightly acidic while the pH of the body fluids is slightly alkaline and unsuitable for their activity.
- After death, lactic acid accumulates in the tissues and cathepsins become activated.

  Some physiological mechanisms can also be explained by cathepsins activation e.g.:

  1- Generalized atrophy of old age (senility).
  2- Involution of uterus after labour.
  3- Mammary glands involution after lactation period.
- When the blood flow to an organ decreases, acidic metabolites accumulate and stimulate cathepsins activity. So, the reson of cathepsin activity in these cases is probably ischaemia of the affected organs.

Regulation of enzyme activity:

— Enzyme activity can be regulated by control of enzyme synthesis. Enzyme synthesis can be controlled by induction or by repression and derepression.

A- Induction of enzyme synthesis: for a substance to be utilized by a cell, it must enter inside the cell at first by an enzyme called permease and then utilized inside the cell by enzyme specific to this substrate.

— On the other hand, if a cell, this means that the enzyme permease for this substrate is absent or the enzyme specific to its utilization inside the cell is absent or both enzymes are cannot utilize a substrate absent.

— But in certain types of bacteria, if a substrate, originally cannot be utilized, is induced to this bacteria during its growth, this substrate has the ability to induce the synthesis of a permease or the enzyme necessary to utilization of this substrate. This phenomenon in enzyme regulation *is called induction*.

- In general, the inducers are substrates for the enzymes or permeases they induce, but in some cases compounds similar to substrates in structure act as inducers but not substrates. These are called *gratitutious inducers*.
- Enzymes whose concentration in a cell does not depend on an added inducer are called *constitutive enzymes*.
- From the genetic point of view, every enzyme can be synthesized by stimulation of its *structural gene*.
- Also it was found that a group of structural genes can be controlled by one *operator gene (open theory)*.
- So if this operator gene is induced by one inducer, all the structural genes controlled by this operator gene can

synthesize all the corresponding enzymes at the same time. This phenomenon is called *coordinate induction*.

B- Repression and derepression of enzyme synthesis:

– In certain types of bacteria capable of synthesis of a particular amino acid, the addition of this amino acid will cause inhibition of synthesis of new molecules of this amino acid.

– This phenomenon is called repression of enzyme synthesis.

- The added amino acid inhibits the synthesis of enzymes responsible for the synthesis of this amino acid and can be called *repressor*.
- At a genetic level, coordinate repression occurs also.
- If the repressor is removed from the medium derepression occurs and the amino acid can be synthesized again.
- This phenomenon is not restricted only to amino acids but can operate to all synthetic pathways in bacteria and can be called *product feedback repression*.
- In another phenomenon, one of the catabolic intermediate product in a metabolic pathway can repress the synthesis of enzymes responsible for such catabolic reaction. This is called *catabolic repression*.

Importance of enzymes in clinical diagnosis

Functional plasma enzymes:

These are enzymes present in blood together with their substrates and have physiological function in blood *e.g.*: lipoprotein lipase, pseudocholine esterase and proenzymes of blood clotting and clot lysis.

Non functional plasma enzymes:

There are enzymes having no specific function in blood and having no substrate to act on it. They are present in low

concentration in blood and originally they are present in different organs inside their cells.

If the cells of these organs are destroyed by any disease, these enzymes are liberated and appear in higher concentration in blood.

So the presence of these non functional enzymes in higher concentration than normal in blood is of great clinical importance for diagnosis of such disease *e.g.*

2) **Lipase** it is elevated in acute pancreatitis and pancreatic carcinoma.
3) **Amylase** it is elevated in parotitis, acute pancreatitis and pancreatic carcinoma.
4) **Trypsin** also in pancreatic diseases.
5) **Cholinesterase** is lowered in exposure to insecticides and is elevated in conditions of active haemopoiesis.
6) **Alkaline phosphatase** is elevated in liver diseases as obstructive jandice or in bone diseases *e.g.* rickets, osteogenic sarcoma and Paget's disease.
7) **Acid phosphatase** is elevated in cancer prostate.
8) **Transaminases:**

   Glutamic pyruvic transaminase or GPT is elevated in destruction of liver cells *e.g.* infective hepatitis.

   Glutamic oxaloacetic transaminase or GOT is elevated in cardiac infarction.

8) **Lactic acid dehydrogenase or LDH** is also elevated in cardiac infraction.
9) **Creatine phosphokinase (CPK)** is elevated in cardiac and skeletal muscle diseases.

CLINICAL IMPORTANCE OF ENZYMES

Enzymes are highly selective tools used by the living organism to carry out the many thousands of interrelated chemical changes in the cell, involving the formation or destruction or interconversion of an extremely wide range of chemical compounds. A knowledge of enzymology is also important to medical students because enzymes are involved in many aspects of health and disease, diagnosis and treatment as:

(1) Inborn errors of metabolism:

Some clinical conditions are now known to be due to a hereditary defect in a particular enzyme. Galactosaemia (high level of galactose in the blood) caused by absence of a key enzyme required to convert this sugar into the more easily metabolised, glucose.

(2) Toxicity:

Many chemicals do their poisonous action to an interference with essential enzymes, *e.g.* cyanide kills by blocking cytochrome oxidase, a vital enzyme for cellular respiration.

(3) Chemotherapy

Some anti-bacterial drugs may owe their effectiveness to an ability to interfere with bacterial enzyme systems without seriously affecting the metabolism of the host.

(4) Vitamins

The majority of vitamins form essential parts of enzyme systems. If the supply of vitamins in the diet is inadequate, defects develop in the enzymic mechanism, leading to serious consequences as seen in deficiency diseases.

(5) Diagnosis

Assay of particular enzymes can sometimes provide useful Information, *e.g.* acute hepatitis (distinct rise in the activity of transaminase in the serum will occur before the more characteristic symptoms of jaundice appear).

Myocardial infarction (a coronary heart attack) causes damage to heart muscle cells and this leads to the release of the cellular enzymes into the blood.

(6) Treatment

A number of application of enzymes in treatment has been suggested, but much of the work is still in the experimental stage. *e.g.* asparaginase in the control of some forms of cancer.

COENZYMES

Coenzymes regarded as second substrates:

– The coenzyme may be regarded as a second substrate or cosubstrate for 2 reasons. First, the chemical changes in the coenzyme exactly counterbalance those taking place in the substrate. For example, in oxidoreduction reactions, when one molecule of substrate is oxidized, one molecule of coenzyme is reduced.

- Similarly, in transamination reactions pyridoxal phosphate acts as a second substrate in 2 concerted reactions and as carrier for transfer of an amino group between different α-keto acids.
- A second reason to accord equal emphasis to the coenzyme is that this aspect of the reaction may be of greater fundamental physiologic significance. For example, the importance of the ability of muscle working anaerobically to convert pyruvate to lactate does not reside in pyruvate or lactate.

- The reaction serves merely to oxidize NADH to NAD^+. Without NAD^+, glycolysis cannot continue and anaerobic ATP synthesis (and hence work) ceases.
- Under anaerobic conditions, reduction of pyruvate to lactate reoxidizes NADH and permits synthesis of ATP.
- Other reactions can serve this function equally well. For example, in bacteria or yeast growing anaerobically, metabolites derived from pyruvate serve as oxidants for NADH and are them selves reduced.

Many coenzymes are derivatives of B vitamins & of adenosine monophosphate:

B vitamins from part of the structure of many coenzymes. The B vitamins *nicotinamide, thiamin, riboflavin* and *pantothenic acid* are essential reductions, and *folic acid* and *cobamide* coenzymes function in one-carbon metabolism. Many coenzymes contain adenine, ribose, and phosphate and are derivatives of adenosine monophosphate (AMP). Examples include NAD^+ and $NADP^+$.

Types of Coenzymes:

I) Coenzymes that transfer hydrogen (hydrogen carriers):

1. NAD and NADP.
2. FMN and FAD.
3. Glutathione.
4. Lipoic acid.
5. Coenzyme Q.
6. L-Ascorbic acid.

Nicotin-amide

Adenine

D-Ribose

D-Ribose

*Phosphate attached here in NADP

Pyrophosphate

Nicotinamide adenine dinucleotide

Pyrophosphate

D-Ribitol

D-Ribose

Adenine

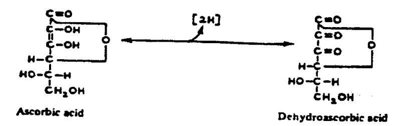
Flavin

Flavin adenine dinucleotide (FAD)

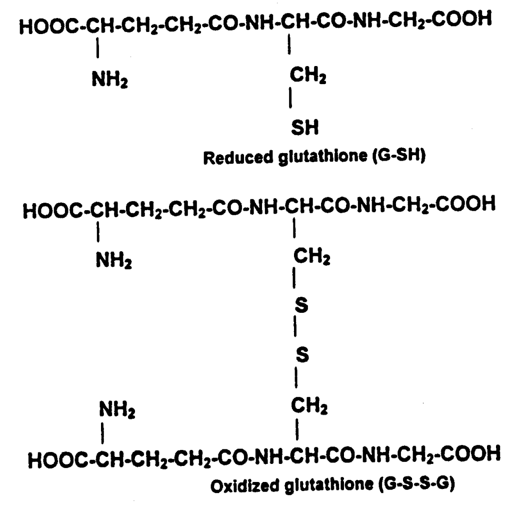
D-Ribitol

Flavin

Flavin mononucleotide (FMN)



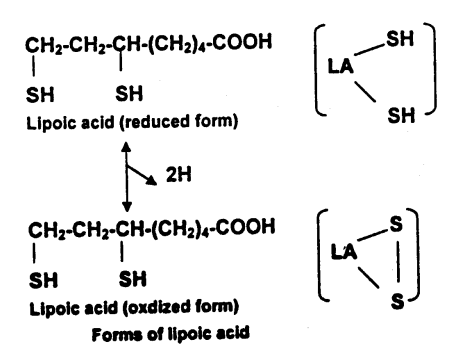
Oxidation of vitamin C



Reduced glutathione (G-SH)

Oxidized glutathione (G-S-S-G)

Glutathione serves as a coenzyme for a number of oxidoreductases, for example, glutatione peroxidase.



Lipoic acid (reduced form)

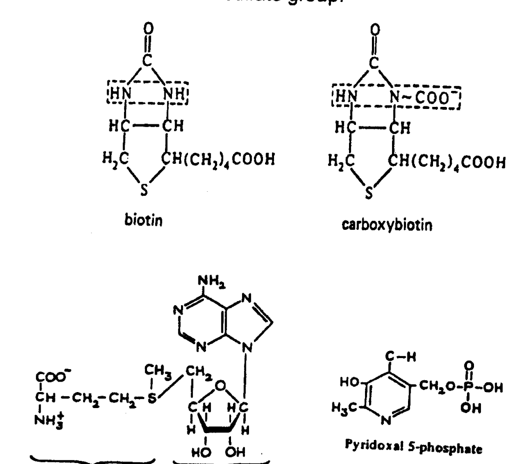
Lipoic acid (oxdized form)

Forms of lipoic acid

Coenzyme Q (CoQ)

II) Coenzymes that transfer groups other than hydrogen:

1. Pyridoxal phosphate: carrier of amino group.
2. Biotin: carrier for CO_2.
3. Coenzyme A: carrier for acetyl and acyl groups.
4. Lipoic acid: carrier for acetyl and acyl groups.
5. S-adenosyl methionine: carrier of CH_3 group = methyl donor.
6. PAPS: carrier of sulfate group.



Pyridoxal 5-phosphate

The structure of S-adenosylmethionine.

Pantothenic acid

Pantoic acid

β-Alanine

Thioethanolamine

Pantetheine

Pyro-phosphate

D-Ribose 3-phosphate

Adenine

Structure of coenzyme A.

3'-phosphoadenosine-5'-phosphosulphate (PAPS)

CATALYTIC NUMBERS OF ENZYMES

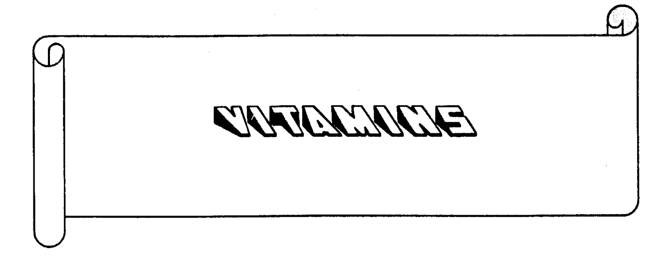
1. Almost all of the chemical changes (absorption, digestion, metabolism, locomotion, putrefaction, etc.) that take place in a living organism are speeded up by enzymes (catalysts). Without these catalysts the reactions proceed too slowly for biological systems to function at any significant rate.
2. A catalyst is defined as an agent which affects the velocity of a chemical reaction without appearing in the final products of that reaction.
3. In an enzyme catalyzed reaction (A + B $\longleftrightarrow$ C + D), the enzyme influences the reaction velocity of both forward and backward reactions to the same extent. However, the direction in which the reaction proceeds is dependant upon mass-law considerations and the availability of free energy.
4. Enzymes also show the typical features of catalysts such as:
   a) not being consumed in the reaction,
   b) being needed only in minute quantitries,
   c) reversible and irreversible inhibition, etc. This is discussed much more fully previously on, in the section on enzyme kinetics.
5. Enzymes are proteins and are produced by living cells. They possess a high degree of specificity (*i.e.*, they usually catalyze only one type of reaction, frequently acting only on one molecular species) and are classified according to the type of reactions they catalyze.
   i) oxidation-reduction.
   ii) transfer of groups.
   iii) hydrolysis of compounds.
   iv) non-hydrolytic removal of groups.
   v) isomerization.
   vi) joining of two molecules with the breaking of a pyrophosphate bond.

The following table is a list consisting of examples of enzymes in the above classes. It is by no means complete with respect to classes, subclasses, or examples.

Classification of enzymes:

Main class and subclasses	Examples
1. Oxidoreductases:	
1.1. Acting on the CH-OH group of donors	
1.1.1. with NAD or NADP as acceptor	Alcohol dehrogenases, lactate dehydrogenase
1.1.2. with O_2 as acceptor	Glucose oxidase
1.2. Acting on the aldehyde or keto-group of donors	
1.2.1. with NAD or NADP as acceptor	Glyceraldehyde-3-phosphate dehydrogenase
1.2.2. with O_2 as acceptor	Xanthine oxidase
1.3. Acting on the CH-CH group of donors	
1.3.1. with NAD or NADP as acceptor	Dihydrouracil dehydrogenase
1.3.2. with a cytochrome as an acceptor	Acyl-CoA dehydrogenase
1.4. Acting on the CH-NH2 group of donors	
1.4.1. with O_2 as acceptor	Amino acid oxidases
2. Transferases	
2.1. Transferring C_1-groups	
2.1.1. Methyltransferases	Guanidoacetate methyl-transferase
2.1.2. Hydroxymethyltransferases and formyltransferases	Serine hydroxymethyl-transferase
2.1.3. Carboxyltransferase and carbamoyltransferase	Ornithine carbamoyl-transferase
2.2. Acyltransferases	Choline acetyltransferase
2.3. Glycosyltransferases	Maltose phosphorylase
2.4. Transferring N-containing groups	
2.6.1. Aminotransferases	Transaminases
2.5. Transferring phosphorus-containing groups	
2.5.1. Phosphotransferases with an alcohol group as an acceptor	Glucokinase
2.6. Transferring sulfur-containing groups	
2.6.1. CoA-transferases	
2.6.1.1. Acetyl-CoA: propionate CoA-transferase	Propionate CoA-transferase

Main class and subclasses	Examples
3. Hydrolases	
3.1. Cleaving ester linkages	
3.1.1. Carboxylic ester hydrolases	Esterases, lipases
3.1.2. Phosphoric monoester hydrolases	Phosphatases
3.1.3. Phosphoric diester hydrolases	Snake venom phospho-diesterase
3.2. Cleaving glycosides	
3.2.1. Glycoside hydrolases	Amylase, β-Glucosidase, etc.
3.2.2. N-Glycoside hydrolases	Nucleosidases
3.3. Cleaving peptide linkages	
3.3.1. α-Aminopeptide amino acid hydrolases	Leucine aminopeptidase
3.3.2. α-Carboxypeptide amino acid hydrolases	Carboxypeptidase
3.3.3. Peptidopeptide hydrolases (=endopeptidases)	Pepsin, trypsin, chymotrypsin
3.4. Acting on C-N bonds other than peptide bonds:	
3.4.1. Urea amidohydrolase	Urease
3.5. Acting on acid anhydride bonds	
3.5.1. ATP phosphohydrolase	ATPase
4. Lyases	
4.1. C-C lyases	
4.1.1. Carboxy lyases	Pyruvate decarboxylase
4.1.2. Aldehyde lyases	Aldolase
4.2. C-O lyases	
4.2.1. Hydrolyases	Fumarate hydratase (=histidase)
4.3. C-N lyases	Histidine-ammonia lyase (=histidase)
5. Isomerases	
5.1. Racemases and epimerases	
5.1.3. Acting on carbohydrates	Ribulose-5-phosphate eimerase
5.2. Cis-trans isomerases	Maleylacetoacetate isomeras
5.3. Intramolecular oxidoreductases	
5.3.1. Interconvertting aldoses and ketoses	Glucosephosphate isomerase
5.4. Intramolecular transferases	Methylmalonyl-CoA mutase
6. Ligases	
6.1. Forming C-O bonds	
6.1.1. Amino acid-RNA ligases	Amino acid-activating enzymes
6.2. Forming C-N bonds	
6.2.1. Acid-ammonia ligases	Glutamine synthetase
6.2.2. Acid-amino acid ligases	Peptide synthetase, glutathione synthetase
6.4. Forming C-C bonds	
6.4.1. Carboxylases	Acetyl-CoA carboxylase


VITAMINS

VITAMINS

DEFINITION:

Vitamins are low molecular weight organic molecules that must be present in small amounts in the human diet to maintain good health. They are required in the diet because they perform essential functions and yet either are not synthesized by humans or are produced in amounts insufficient to support normal metabolism.

(Vit = life. Amin = amines essential for life)

Complex organic compounds which must be present in food in small amounts to enable growth; health and life to be maintained. Most of them can be prepared now synthetically.

Action: mainly as coenzymes specially water soluble or part of its structures. Vitamin deficiency interferes with their respective actions leading to impaired synthesis or accumulation of certain metabolities. Not always possible to correlate functions with disturbance due to deficiency.

Provitamins:

These are vitamin precursors which are converted to vitamins inside the body e.g.

- Carotenes are provitamin A.
- 7-Dehydro-cholesterol is provitamin D_3.

Vitamer:

- When a vitamin is present in more than one chemical formula, each of them is called a vitamer e.g.

  Vitamin A has 2 vitamers: A_1 and A_2.

  Vitamin D has 2 vitamers: D_2 and D_3.

  Vitamin E has 2 vitamers: α, β, γ and δ.

Vitagens:

These include both essential amino acids and essential fatty acids.

VITAMIN DEFICIENCIES

1. **Single:** giving rise to specific manifestation.
2. **Multiple:** more than one vitamin.

I. Multiple Deficiency Disorders:

More common than single deficiency. One vitamin deficiency may be presenting but others although present may not be clinically evident.

Treatment: Primarily by diet to provide more than one vitamin and vitamins from natural sources. Synthetic vitamins additional.

II- Sub-clinical Deficiency Disorders:

Symptomes: Vague, Fatigue, Lassitude, Loss of appetite, Irritability, Negative laboratory findings.

Treatment: Proper diet. Avoid drugs.

Causes:

1. Deficient intake.
2. Secondary to other diseases: Excossive loss (B deficiency in polyuria of diabetes mellitus), Defective absorption (steatorrhea and chronic diarrhea). Defective storage and utilization (vitamin K deficiency in liver disease).
3. Relative deficiency: Pregnancy, Growth, Fevers, Infections, Diabetes mellitus, Hyperthyroidism.

Diagnosis:

Clinical features, blood and urine tests. Therapeutic tests.

ANTI-VITAMINS

Definition:

Substances interfering with availbility or action of vitamins:

1. Structural analogues (vitaminers):

Chemically closely related but no physiological action. Compete with vitamin. Synthetic. *Example*: Pyrithiamine for thiamine.

2. Enzymes:

Destroy vitamins. *Example*: thiaminase (against thiamin) and oxidative destruction of vitamin A (vitamin E protects against it).

3. Binding vitamins:

Vitamin unabsorbable or inffective. *Example:* avidin binding biotin in egg white.

4. Competition:

Sulphonamide drugs competition with para-aminobenzoic acid in folic acid bacteria-forming in the gut.

VITAMINS AS DRUGS

- This fact has got pharmacological basis.
- Vitamins must be used in big doses.

1. **Thiamine**: Anti-neuritic.
2. **Vitamin E**: Muscular dystrophies, Habitual abortion, Ischaemic heart disease, Lipotropic, Antiageing.
3. **Ascorbic acid**: Rheumatic fever, Influenza, Common cold.
4. **Vitamin B_{12}**: Diabetes mellitus. Myocardial and cerebral diseases. Peripheral neuropathy.
5. **Vitamin D (calciferol)**: Lupus vulgaris. Hypoparathyroidism.

CLASSIFICATION OF VITAMINS

On the basis of their solubility, vitamins are classified into two groups:

A) Water soluble vitamins.

B) Fat soluble vitamins.

	Fat sol. vitamin	Water sol. vitamin
Chemical composition	Only C-H-O	A long with C-H-O The group contain N-S-Co.
Occurrence	-In plant as pro-vitamins precursors which converted to vitamin in the body	No pro-vitamins
Physiological action	-Regulation the metabolism of structural units. -Each one have one or more specific independent role.	-Collectively occurred with energy transfer in every cells. -Acted as Co-enzymes
Absorption	-Absorbed with fat so related to factors governed fat absorption	-Absorbed by simple processes as there is a content absorption of water from intestine
Storage	-Stored whenever fat is deposited. -The amount absorbed related to the intake.	-Are not stored in the same way or to the same extent
Excretion	-Usually through stool	-Generally the excretion following metabolic use in urine. -B-vitamins also present in stool.

A) Water Soluble Vitamins

- They are members of the B-complex and vitamin C.
- All of them are polar, hydrophilic molecules.
- They are readily excreted in urine once their concentration exceeds the renal threshold; thus toxicities are rare while deficiencies are common and frequently occur in the form of a multiple vitamin deficiency state.

- Nevertheless, definite syndromes are characteristic of deficiencies of specific vitamins *e.g.*: beriberi (thiamin deficiency); cheilosis, glossitis, sebonhea and photophobia (riboflavin deficiency); pellagra (niacin deficiency); periphenl neuritis (pyridoxine deficiency); megaloblastic anaemia. methyl malonic aciduria, and pernicious anaemia (cobalamin deficiency); megaloblastic anaemia (folic acid deficiency); and scurvy (ascorbic acid deficiency).
- All, But one (cobalamin) can be synthesized by plants.
- Most of them are converted to coenzymes, which are utilized either in the pathways for energy generation or hematopoiesis.

I. B-COMPLEX VITAMINS:

- Stable in acids.
- Unstable in alkaline.
- Heat stable except B_1.
- Distory by hight except Niacin.

Human nutritionally essential B-vitamins are:

a) Energy releasing B-complex vitamins:
   1. Thiamin (vitamin B_1).
   2. Riboflavin (vitamin B_2).
   3. Niacin (nicotinic acid, nicotinamide) (vitamin B_3).
   4. Pantothenic acid (vitamin B_5)
   5. Vitamin B_6 (pyridoxal, pyridoxine, pyridoxamine).
   6. Biotin.
   7. Lipoic acid (thoctic acid).

B) Hematopoietic vitamins:
   1. Vitamin B_{12} (cobalamin).
   2. Folic acid (pteroylglutamic acid).

Common sources of vitamin B complex:

Nearly same for all members of vitamin B complex.

1- Plant sources: grains, cereals, yeast and green leafy vegetables.

2- Animal sources: liver, kidney, egg yolk and fishes.

3- Intestinal flora.

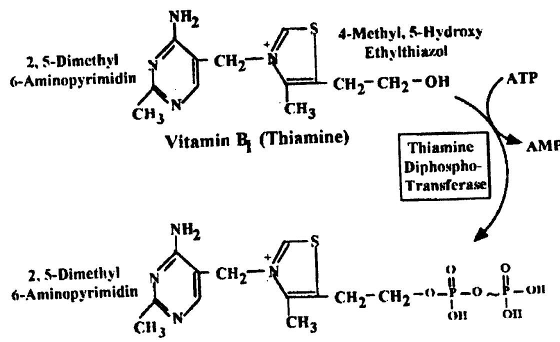
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1. Thiamin (vitamin B_1): → Anti-Beri Beri

- Firstly discovered by the Poland chemist casimir Funk at (1912) as Anti-Beri Beri factor from rice polish.
- There is an ancient error trust that administration of vitamin B_1 prevent mosquites bits which may be due to vitamin B_1 relieves the skin itching.

Chemistry:

- It is a basic substance formed of:
- a pyrimidine ring.
- a thiazole ring connected together through a methylene group.



Thiamine Pyrophosphate (Active Form)

Properties:

- It is a thermolabile white crystalline substance soluble in water and ethyl alcohol.
- It isstable in acid solution but destroyed by alkalies and light.
- Alcoholic in nature.
- Sulphur containing vitamin.
- The alcoholic group on thiazole ring reacts with phosphoric acid to form ester called thiamine pyrophosphate (TPP) acting as a coenzyme (cocarboxylase).
- Thiamine or its pyrophosphate is oxidized by mild oxidizing agents e.g. potassium ferricyanide and results in the formation of thiochrome.

Requirements: 1-1½ mg/day.

Thiamin is a white, crystalline compound, readily soluble in water. It has the odor and flavor characteristic of yeast.

In cooking, thiamin is destroyed to any great extent if the temperature is not much above 100°C and if the heating is not continued for too long a time.

Occurrence (Sources):

- Unrefined cereal grains (the milling of wheat flour has lowered its thiamin content more than 80 per cent.
- Meats and yeast although the later is consumed in relatively small its content of thiamin is high.
- Milk, fresh fruits and vegetables are low in thiamin but consumed in relatively large amount.

Functions:

- Conversion of thiamin to its active form thiamin diphosphate (pyrophosphate) occurs in brain and liver by an ATP-dependent thiamin diphosphotransferase.

Thiamin diphosphate (TPP) serves as a coenzyme transferring an activated aldehyde unit in the following enzymatic reactions:

1. Oxidative decarboxylation of α-keto acids (*e.g.* a-ketoglutarate, pyrivate and the α-keto analogs of leucine, isoleucine, and valine).

Pyruvic $\xrightarrow[\text{Lipoic, Mg}]{\text{TPP, CoASH, NAD}}$ **active acetate**

Simple decarboxylation

Pyruvic $\xrightarrow{\text{TPP}}$ **Acetaldhyde**

Xylulose $\xrightarrow[\text{Transketolase}]{\text{TPP}}$ **Phosphoglycerate**

2. Transketolase reactions (*e.g.*, in the pentose phosphate pathway).
3. TPP plays an important role in the transmission of nerve impulse and it appears to be required for acetylcholine synthesis.
4. Coffe and tea contain substances destroy vitamin B_1.

Deficiency manifestations:

Deficiency of vitamin B_1 results in deficiency of TPP which leads to:

1- Accumulation of pyruvic acid in blood due to decreased activity of oxidative decarboxylation.

2- Increase of lactic acid in blood.

3- Appearance of methyl glyoxal in urine due to decreased activity of glyoxalase enzyme catalyzing the conversion of methyl glyoxal to lactic acid in the liver.

4- Accumulation of pentose sugars in R.B.C's as a result of retardation of transketolase reaction.

- Severe thiamin deficiency (beriberi): It is caused by carbohydrate rich/low thiamin diets:
  - Dry beriberi is characterized by advanced neuromuscular symptoms, including atrophy and weakness of the muscles.
    - Atrophy of prepheral nerves.
    - Loss of sensation
  - Wet beriberi: the previous symptoms (dry beriberi) are coupled with edema.
    - Congestive heart failure.
    - Prepheral neuritis.
  - In pigeon paralysis of neck muscles-head drown back-The breast is pointed straight up in stargaizing attitude.

Vitamin B_2 "Riboflavin"

Chemistry:

It an orange yellow compound formed from:

- Flavin pigment or dimethyl isoalloxazine.
- D-ribitol derived from D-ribose.

Properties:

- Orange yellow coloured vitamin.
- It gives green yellow fluorescence in ultraviolet rays.
- It undergoes reversible reduction to a colourless substance called lecucoriboflavin.
- Alcoholic in nature due to ribitol.
- Its pure crystalline form is slightly soluble in water.

- Heat stable in neutral or acid solution but not in alkaline solution.
- Destroyed by light.
- On exposure to light, the ribityl residue splits off with the formation of a yellow pigment soluble in chloroform which is called.
  a- Lumiflavin (in alkaline medium) or
  b- Lumichrome (in acid or neutral medium).
- Riboflavin is present in tissues as flavin mono-nucleotide "FMN" and flavin adenine dinucleotide "FAD" coenzymes.

Occurrence:

It occurs widely in nature.

- Milk is an important source (lactoflavin one of the pigments of milk is identical with riboflavin).
- Excellent sources are meats especially liver and kidney, fish and eggs.
- Leafy vegetables and fruits contain moderate quantities.

Functions:

Flavin mononucleotide (FMN) is formed by ATP-dependent phosphorylation of riboflavin, whereas flavin adenine dinucleotide (FAD) is synthesized by a further reaction with ATP in which the AMP moiety of ATP is transferred to FMN. Biosynthesis of FMN and FAD seems to occur in most tissues.

Riboflavin mononucleotide (FMN)

Flavin adenine dinucleotide (FAD)

FMN and FAD serve as prosthetic groups of oxidoreductase enzymes (flavoprotein enzymes).

Flavoprotein enzymes function in:

1. Oxidative decarboxylation of α-keto acids FAD which act as H_2 carrier in oxidative-reduction reactions is the coenzyme of dihydrolipoyl dehydrogenase).
2. Oxidative degradation of fatty acids (FAD is the posthetic group of acyl CoA dehydrogenase).
3. Oxidative deamination of α-amino acids; one L-amino acid oxidase specific for L-arnino acid contains tightly bound FMN as the prosthetic group, and the other is D-amino acid oxidase specific for D-amino acid contains FAD as prosthetic group.
4. Dehydrogenation of succinic acid in the citric acid cycle (succinate dehyrogenase with FAD as a prosthetic group).
5. Catalize reactions of molecular oxygen as xanthin oxidase to produce uric acid.
6. Warburg's yellow enzymes (B_2 is them Co-enzyme).
   - L-amino acid oxidase (FMN).

- D-amino acid oxidase (FAD).
- Histaminase.
- Cytochrome reductase.
- Succinic dehydrogenase.
- Xanthin reductase.

7. B_2 is necessary for protein biosynthesis and growth.
   - High intake inhibit the appearance of choline induced tumor in rats.

In their role as coenzymes, flavoproteins undergo reversible reduction of the isoalloxazine ring to yield the reduced forms $FMNH_2$ and $FADH_2$.

Reduction of isoalloxazine ring in flavin nucleotides

Deficiency:

1- Cheilosis is a dry fissured lips.

2- Angular stomatitis is fissuring at the angle of the mouth.

3- Seborrhoeic dermatitis in the face.

4- Vascularization of cornea, dryness and photophobia.

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3. Niacin (Nicotinic acid):

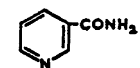
- Anti-Black tongue in Dogs.
- Anti-Pellagra (pellagra preventing factor).

Chemistry:

Nicotinic acid is a monocarboxylic acid derivative of pyridine.



Niacin (nicotinic acid)



Niacinamide (nicotinamide)

When pure it occurs as white, needlelike crystals, which are water soluble and stable in air and also to heat.

- There is little loss in cooking unless the "cook water" is discarded.
- It is absorbed in the intestine asnicotinate but the largest portion is excreted as N-methyl nicotinamide.
- Some niacin could be synthesized from tryptophan by plants and most animals and this pathway requires pyridoxal phosphate.

However the conversion of tryptophan to niacin is relatively inefficient (60 mg of tryptophan are required for the production of 1

mg of niacin) and occurs only after all of the body requirements for tryptophan (protein synthesis and energy production) have been met. Thus in practical terms, most people require dietary sources of both tryptophan and niacin.

— The protein of corn (zein) is virtually free of tryptophan the pellagra resulted by high corn (low protein) diet.

Occurrence:

— The major sources of niacin are:

- Tryptophan containing proteins such as lean meats especially liver, fish and eggs.
- Food stuffs containing nicotinic acid per se; unrefined grains and cereals, yeast, milk and leafy vegetables.

N.B.: Some niacin could be synthesized from tryptophan by plants and most animals and this pathway requires pyridoxal phosphate, but this conversion is inefficient and most people require dietary sources of both tryptophan and niacin.

Requirements: 13-19 mg/day

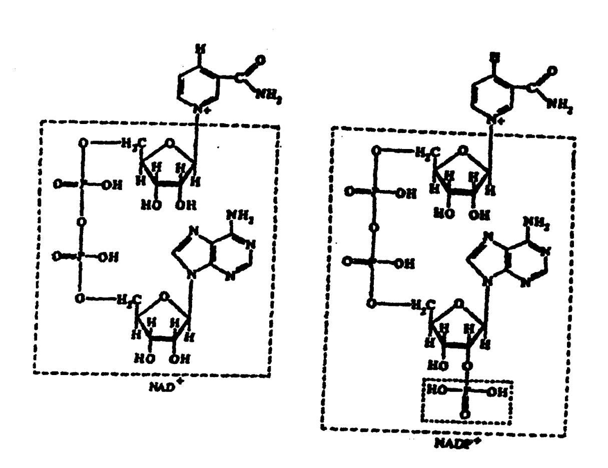
Function:

— Nicotinate is the turn of niacin required for the synthesis of NAD^+ (nicotinamide adenine dinucleotide) and $NADP^+$ (nicotinamide ademine dinucleotide phosphate).

— NAD^+ and $NADP^+$ are coenzyme of many oxidoreductase enzymes occurring both in cytosol and within the mitochondria.

— Generally, NAD-liked dehydrogenases catalyze oxidoreduction reactions in oxidative pathways, *e.g.* the citric acid cycle.

— Whereas NADP-linked dehydrogenases or reductases are often found in pathways concerned with reductive synthesis *e.g.* the pentose phosphate pathway.



The mechanism of oxidoreduction involves a reversible addition of a hydride ion (H^+) to the pyridine ring plus the generation of a free hydrogen ion (H^+).

$$NAD^+ + AH_2 \longrightarrow NADH + H^+ + A$$

Reduction of NAD$^+$

Examples of dehydrogenase enzymes that utilize NAD^+.

- Lactate dehydrogenase.
- Glyceraldehyde-3-phosphate dehydrogenase.
- L-malate dehydrogenase.
- Isocitrate dehydrogenase (the NAD^+ -linked isocitrate dehydrogonase is found only in mitochondria It is an allosteric enzyme stimulated by ADP).

Examples of $NADP^+$ linked dehydrogenases

- Isocitrate dehydrogenase (it occurs both in mitochondria and cytosol, and it does not appear to be an allosteric enzyme).
- Glucose-6-phosphate dehyrogenase.
- Glutathione reductase.
- Malic enzyme (NADP malate dehydrogenase)

Dehydrogenase that may use either pyridine nucleotide Coenzymes:

- L-glutamMe dehyrogenase.

Deficiency:

– Borderline deficiencies of niacin are manifested by glossitis.

– Pronounced deficiencies lead to pellagra (pella = skin, agra = rough) which is characterized by "*the three Ds*": dermatitis, diarrhea and dementia *(lack of concentration)* in dog [canine Black tongue disease] as dark-red area with necrotic lesions in mucosa of mouth.

- Dermatitis is usually seen in skin areas exposed to sun light and is symmetric.
- It is in the form of pigmentation and thickening of the skin.
- The neurologic symptoms start by nervous disorders and mental disturbances, in the latter stages dementia occur.

- Pellagra is seen in patients with severe malabsorption problems, in malignant carcinoid syndrome, in Hartnup disase and in elderly on very restricted diet.

Extretion:

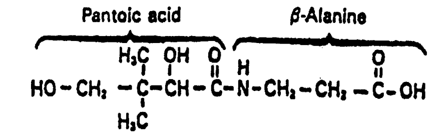
- In urine: as nicotinamide or nicotinic acid or N_1 methyl nicotinamide.
- In sweat: as nicotinamide (traces).
- In milk: as nicotinamide (small amounts).

4. Pantothenic Acid: (chick anti dermatitis factor)

Due to it is found in all living tissues it named pantothen = in all place.

Chemistry:

Pantothenic acid is an amide of pantoic acid and β-alanine.



Pantothenic acid

Requirements: 5-10 mg/day.

Occurrence:

- It is very widespread in natural foods specially Royal Jelly being particularly abundant in animal tissues and products (liver, kidney, milk, eggs), whole-grain cereals and legumes (cabbage and cauliflower).

Functions:

- Pantothenic acid is an essential component of CoA and phosphopantetheine of fatty acid synthase and thus is required for the metabolism of all fat, protein, and carbohydrate.

Biosynthesis of CoA: Pantothenic acid is absorbed readily in the intestine and subsequently phophorylated by ATP to form 4-phosphopantothenic acid. Then cysteine is added to the phosphopantothenic acid, and the carboxyl group of cysteine is subsquenthy removed, resulting in the net addition of thio ethanolamine to phosphoeantothenic acid, generating 4-phosphopantetheine.

Active pantothenic acid is coenzyme A (CoA) and the acyl carrier protein (ACP). 4-phosphopantetheine-generated from pantothenic acid is the prosthetic group of both CoA and ACP

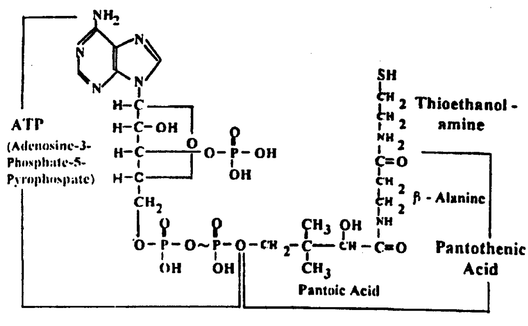
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      H3C OH  O            O
        |  |  ||  H        ||  H
CH2-C-CH-C-N-CH2-CH2-C-N-CH2-CH2-SH
|     |
|    H3C
O
|
O=P-OH
  |
  OH
```

4-Phosphopantetheine

The thiol group (-SH) in both CoA and ACP acts as a carrier of acyl radicals (R-CO^{-}) in enzymatic reactions involved in:

- Fatty acid oxidation and synthesis (ACP participates in reactions concerned with fatty acid synthesis).
- Combine with acetate to form acetyl co-A.
- Oxidative decarboxylation of α-ketoacids.
- Acetylation reactions as:
    - Formation of acetylcholine where the acetyl radical is attached to the acceptor (choline) at the carbonyl end reaction).
- Formation of citric acid where the acetyl radical is attached to the acceptor (oxloacetate) at the methyl end (tail reaction).

- Synthesis of Adrino cortical hormone.
- Cholesterol synthesis.
- Detoxication of sulphonamides.
- Combine with succinic to form succinyl CoA necessary for Hb synthesis.
- Important for F.A. oxidation.



Co A SH

Deficiency:

Deficiency of pantothenic acid is shown in the following symptoms:

1- Lesions of the skin and its appendages.
2- Normocytic normochrine anaemia.
3- Impairment of adrenal function.
4- Gastro-intestinal (GIT) disturbances.
5- In rats: It causes dermatitis especially around the eye "spectacle eye" and depigmentation of hair "antigrey hair vitamins".

In animals:

a) General effects:
    1. Inadequate growth.
    2. Decreased reproductive activity.

b) Changes in skin (hens and rats).
   Hair: Dermatitis and loss of hair.

c) Fatty liver.

d) Nervous manifestations *e.g.* myelin degeneration of peripheral nerves.

e) Gastro-intestinal manifestations.

f) Haemorrhage below the skin, kidneys and adrenal cortex

Requirements: 5-12 mg/2500 calories.

Determination: Microbiological method: It measures the degree of growth of lactobacillus arbinosus or increase in lactic acid production.

---

5. Pyridoxine (Vitamin B_6): [Anti-Rat dermatitis Acrodermyia]

Chemistry:

Vitamin B_6 consists of 3 closely related pyridine derivatives: pyridoxine, pyridoxal, and pyridoxamine. All 3 have equal vitamin activity, and are naturally occurring forms of vitamin B_6.

Pyridoxine

Pyridoxamine

Pyridoxal

Pyridoxal phosphate

Requirements: 2 mg/day.

Sources: Plant → yeast.
→ rice polishings.
→ germinal portion of various seeds.

Animals → Royal Jelly.
→ egg yolk

Functions:

All forms of vitamin B_6 are absorbed from the intestine. Most tissues contain the enzyme pyridoxal kinase which catalyzes the phosphorylation by ATP of all forms of the vitamin to their respective phosphate esters.

Only pyridoxal phosphate and pyridoxamine phosphate are active as coenzymes.

Phosphorylation of pyridoxal by pyridoxal kinase to form pyridoxal phosphate.

Pyridoxine coenzymes function in a large number of different enzymatic reactions, the most common types of these reactions are:

1. Transaination reactions:

Being cotransaminase it is essential for energy production from amino acids.

2. decarboxylation reactions of amino acids:

Removal of CO_2 from amino acids form corresponding amine. Thus, it is required for synthesis of some neurotransmitters.

* Tryptophan → 5-hydroxytryptophan $\xrightarrow[PLP]{\nearrow CO_2}$ 5-hydroxytryptamine

* Histidine $\xrightarrow[PLP]{\nearrow CO_2}$ histamine

* Glutamate $\xrightarrow[PLP]{\nearrow CO_2}$ γ-amino butyrate (GABA)

Concerned with CNS metabolism

* Tyrosine ——→ dopa $\xrightarrow[PLP]{\nearrow CO_2}$ dopamine → adrenaline and nor-adrenaline

3. Coenzyme for deaminases or dehydrotases of serine and threonine:

Serine (CH_2OH–$C(H)(NH_2)$–$COOH$) $\xrightarrow{-H_2O}$ [CH_2=$C(NH_2)$–$COOH$ ⇌ CH_3–C=NH–$COOH$] $\xrightarrow{H_2O,\ -NH_3}$ CH_3–C=O–$COOH$ (Pyruvic acid)

SERINE DEHYDRATASE

4. Threonine aldolase is also PLP dependent:

H_3C–$C(H)(OH)$–$C(NH_2)(H)$–$COOH$ (L-Threonine) $\xrightarrow{\text{THREONINE ALDOLASE}}$ H–$CH(NH_2)$–$COOH$ (Glycine) + H–$C(=O)$–CH_3 (Acetaldehyde)

5. Pyridoxal phosphate is required for the synthesis of δ-amino levulinic acid, a precursor of heme.
6. The generation of niacin from tryptophan is PLP dependent, hence pellagra is a frequent accompaniment of pyridoxine deficiency byactivation of Kynureninase enzyme.
7. Pyridoxal phosphate is also an essential component of the enzyme glycogen phosphorylase (hence it is essential for energy production).
8. It is essential for transformation of linoleic acid to arachidonic.
9. Transaminase help in conversion of oxalate to glycine so prevent accumulation of oxalate.
10. Inter in the synthesis of many important neurohormones.
11. Trans-sulfuration methionine → serine.
12. desulforation (removal of sulfar) → cysteine → pyruvic

Deficiency:

Causes of deficiency →
- low vitamin intake.
- broad spectrum antibiotics.
- pregnany
- malabsorption and alcoholism.

- Being energy releasing vitamin, some of the symptoms of severe B_6 deficiency are similar to those of the other energy releasing vitamins (skin lesions resembles those of B_2 and niacin deficiency).
- Being essential for the synthesis of some neurotransmitters, these effects are thought to explain:
  - The irritability, nervousness and depression seen with mild deficiencies.
  - Peripheral neuropathy and convulsions observed with severe deficiencies.

- It is essential for heme biosynthesis, its deficiency occasionally cause sideroblastic anaemia (a microcytic anaemia seen in the presence of high serum iron).
- In chicken: - Acute convulsion.
  - Flatter on the pan.
  - Kicking and generally die.
- Adult birds: - Reduced hatchability.
- In animals: - Pellagra due to disturbance of nicotinic acid formation.

N.B.: Isonicotinic acid hydrazide (INH) which is used for treatment of T.B. cause symptoms of B_6 deficiency.

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6. Biotin: (Vitamin H and Co-enzyme-R)

- Anti egg-white injury
- A crystalline growth factor for yeast was isolated from egg-yolk at 1936 was named Biotin.

Chemistry:

It is a heterocyclic sulphur containing vitamin, consisting of fused imidazole and thiophene rings.

```
          O
          ‖
          C
        ╱   ╲
    H-N       N-H
      |       |
    H-C───────C-H
      |       |
    H₂C       CH-(CH₂)₄COOH
        ╲   ╱
          S
```

Properties:

- It is soluble in hot water and dilute alkali.
- Avidin, a basic protein present in raw egg white, forms a very stable biologically inactive complex with biotin.

Sources:

- Egg yolk, liver, kidney, milk and yeast.
- Large amounts are present in Royal Jelly of bees.
- In man much of the biotin requirement is supplied by its synthesis from <u>intestinal bacteria</u>.

Excretion: - By urine and stools.

Requirements: 100-300 μg/day

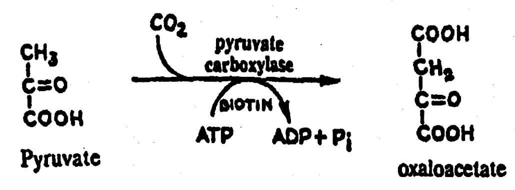
Functions:

Formation of biocytin that functions as the coenzyme for a number of carboxylases which catalyze CO_2 fixation or carboxylation. Examples of these biotin dependent carboxylases:

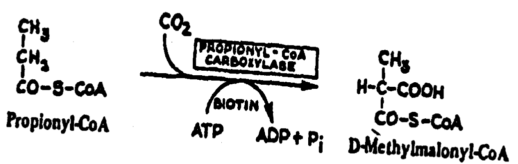
1. Acetyl CoA carboxylase which converts acetyl CoA to malonyl CoA, an important step in the extramitochondrial biosynthetic pathway for fatty acids:

$CH_3 - CO{\sim}S - CoA$ (Acetyl-CoA) + CO_2 —(acetyl-CoA carboxylase, BIOTIN; ATP → ADP + P_i)→ $HOOC - CH_2 - CO{\sim}S - CoA$ (Malonyl-CoA)

2. Pyruvate carboxylase is another biotin dependent en~rme. Pyruvate is converted to oxaloacetate, an important step in gluconeogenic pathway and for replenshirnent of citric acid cycle.



3. Propionyl CoA carboxylase converts propionate to succinate, which can then enter the citric acid cycle.



4. Fixation of CO_2 to form C_6 of purins.
5. Catalyzing the formation of carbamyl phosphate from CO_2 and ammonia.

Deficiency:

- Deficiency can be induced in animals by feeding them oral antibacterial drugs, thus reducing the intestinal flora.
- Biotin deficiency has been produced in man by a low biotin diet containing large amounts of raw egg white, which contains a heat labile protein, avidin. Glycoprotein m.w. 70,000 consists of four identical subunits each of 128 residues of Known sequence each one combine with Biotin denaturation by heat (cocking) abolishes that Biotin-binding capacity that binds biotin in a non digestible form preventing its absorption.
- The symptoms that occurred were: fine scaly desquamation of the skin dermatitis, anaemia, pall skin anorexia, nausea, lassitude and muscle pains.

7. **Lipoic acid:** was observed firstly isolated by Reed (1951) who obtained 30 mg of lipoic acid Coenzyme from 10 tons of beef liver a true Herculean achievement.

Chemistry:

- Thioctic acid (6, 8 dithiooctanic acid)
- It is 6,8-dithio-octanic acid, *i.e.* it is sulphur containing vitamin.

- It occurs in two interconvertable forms; an oxidized form with a cyclic disulfide and a reduced open chain form *"dihydrolipoic acid"* with two SH groups in the 6 and 8 positions.

$$\underset{SH}{CH_2}-CH_2-\underset{SH}{CH}-(CH_2)_4-COOH \xrightleftharpoons{2H} \underset{S}{CH_2}-CH_2-\underset{S}{CH}-(CH_2)_4-COOH$$

Lipoic acid (reduced form) **Lipoic acid (oxidized form)**

Sources:

- It is found in many biological materials including yeast and liver.

Functions:

1- The active coenzyme lipoamide transfers both hydrogen atoms and acyl groups.

2- It functions as one of the coenzymes in the oxidative decarboxylation of pyruvate and other α-ketoacids.

In these reactions *example* oxidative decarboxylation of pyruvate; lipoic acid is bound to the enzyme dihydrolipoyl trans acetylase with transfer of electrons and the acyl group to yield 6-acetyl dihdro lipoic acid.

$$\begin{array}{l} CH_2-SH \\ | \\ CH_2 \\ | \\ CH-S-\underset{\underset{O}{\|}}{C}-CH_3 \\ | \\ (CH_2)_4 \\ | \\ CO \\ | \\ \text{Enzyme}-NH \end{array}$$

6- Acetyl dihydrolipoic acid (linked to the enzyme dihdrolipoyl transacetylase)

3- Lipoic acid is regenerated following transfer of the acetyl group to Co-A and reoxidation of the thiol groups by transfer of electrons to NAD^+ to yield the oxidized or cyclic disulfide form of lipoic acid.

4- Lipoic acid is covalently bound via an amide linkage to the - amino group of specific lysine residue in dihydrolipoyl trans acetylase, forming the lipollysine residue which is also known as lipoamids.

5- Lipoic acid can regenerate vitamin C from its oxidized form dehycroascorbic acid.

6- Lipoic acid increases the levels of glutathione (a very important antioxidant normally found in cells and responsible for mopping up al types of toxins and free radicals). Glutathione supplements are not helpful since glutathione does not have the ability to cross cell membranes. Fortunately lipoic acid can stimulate the production of this antioxidant. This particularly important during periods of excessive stress or exposure to radiation or toxic substances.

7- Lipoic acid also acts as a powerful antioxidant in the brain and is likely to protect brain cells fro toxins.

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8. **Folic acid (Folacin = pteroyl glutamic acid)**

- This nutritional factor was discovered by that yeast cured a nutritional cytopenia induced in monkeys reared on corn containing ration of the type that produced black tongue is dog.

Chemistry

- It is formed of pteridine nucleus (bicyclic nitrogenous compound), ρ-amino benzoic acid (PABA), and glutamic acid.

- Animal cells are not capable of synthesizing PABA or of attaching the first glutamate to pteroic acid, but bacteria and plants can. Thus animals require folic acid in their diet.

COOH
CH – NH – C
Glutamic Acid
CH 2
CH 2
COOH

Para-Amino Benzoic Acid

Pteridine

Pteroyl (Pteroic Acid)

Folic Acid

Aminopterin

Foalate Reductase

Amethopterin

2 NADPH+ H+

2 NADPH+

COOH
CH – NH – C
Glutamic Acid
CH 2
CH 2
COOH

Para-Amino Benzoic Acid

Pteridine

Pteroyl (Pteroic Acid)

Tetrahydrofolic Acid

Requirements: 0.2 mg (200 μg)/day.

Sources:

The major source is leafy vegetables especially spinach other good sources are yeast, Cauliflower, Liver and Kidney.

Functions:

Formation of the important coenzyme tetrahydrofolic acid = FH_4, in liver by help of vit. C to give 6, 7, 8 tetra hydro folic acid is accomplished by folic acid reductase enzyme that needs NADPH + H^+ (reduced Co II) as a hydrogen donor. FH_4 (H_4 folate is the coenzyme for one carbon metabolism.

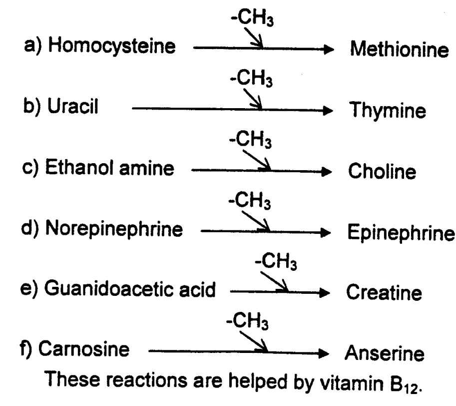
The *"one carbon"* moiety carried on FH_4 may be: methyl ($-CH_3$) methylene ($-CH_2-$), methenyl (-CH=), formyl (-CHO), or formimino (-CH = NH) moiety.

Sources of the one carbon group:

1. Beta carbon of serine, is considered the major source.
2. Formimino group of formimino glutamate (produced during histidine catabolism).
3. Glycine.
4. Formate (*e.g.* intermediary metabolism of tryptophan tbrough kynurenine pathway).

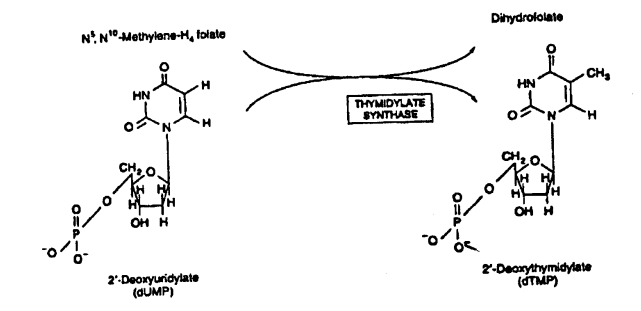
Utilization of single-carbon moiety:

- Intermediary metabolism of some amino acids as glycine, serine, methionine, and histidine.
- Purine biosynthesis (formation of carbon (2) and carbon (8) of purine ring).
- It supplies the β-carbon of serine.
- The methyl group is used for transmethylation reaction *e.g.*



These reactions are helped by vitamin B_{12}.

- Since folic acid is used for reactions leading to synthesis of purines and pyrimidines, so folic acid has a fundamental role in growth, and reproduction.
- So it is necessary for synthesis of red cells and white cells.
- It is necessary for phosphlipid metabolism.
- Synthesis of deoxythymidylic acid (dTMP).



Deficiency:

Folate deficiency may be true (primary), or it may be secondary to B_{12} deficiency. Folate deficiency leads to:

1. Macrocytic anaemia associated with megaloblastic changes in bone marrow.
2. Inhibition of DNA synthesis due to decreased availability of purines and dTMP, slows down the maturation of red blood cells, causing production of abnormally large "macrocytic" red blood cells with fragile membranes impaired growth.
3. Glossitis and gastrointestinal disturbances.

Folic acid antagonist:

These are substances used in treatment of malignant diseases (as cancer) *e.g.* methotrexate (amethopterin) and aminopterin. They act by blocking snythesis of nucleic acids in

malignant cells presumably by preventing the reduction of folic acid to tetrahydrofolic acid as it inhibits folate reductase enzyme.

9. Cobalamin (Vitamin B_{12}):

- Anti- pernicious anemia factor (extrinsic factor) was isolated in 1948 from liver as red crystalline compared containing cobalt and phosphorus.
- Can be obtained as a product of fermentation by stryptomyces griseus.

Chemistry:

It has two characteristic components:

- The central portion of the molecule (corrin ring) consists extensively substituted pyrrole rings.
- It is very similar to porphyrin structure but differs from porphyrin in that 2 of the pyrrole rings (ring I and Iv) are joined directly rather than through a methene bridge.
- Coordinated to the four inner nitrogen atoms of the corrin ring is an atom of cobalt.
- The second component of vitamin B_{12} is 5,6-dimethyl benzimldazole riboside; its nitrogen atom is coordinately bound to cobalt and at the other end from the ribose moiety through phosphate and amino propanol to a side chain on ring IV of the tetrapyrrole nucleus.
- Cobalt is in a coordination state of six, in the rema~g position, it is coordinated to one of several different ligands: cyanide (CN), hydroxy (OH), methyl (CH_3), or 5'-deoxy adenosine to give in order:
    - Cyanocobalamjn.
    - Hydroxycobalamin.
    - Methyl cobalamin.
    - 5'-dcoxyadenosyl cobalamin

Vitamin B_{12}

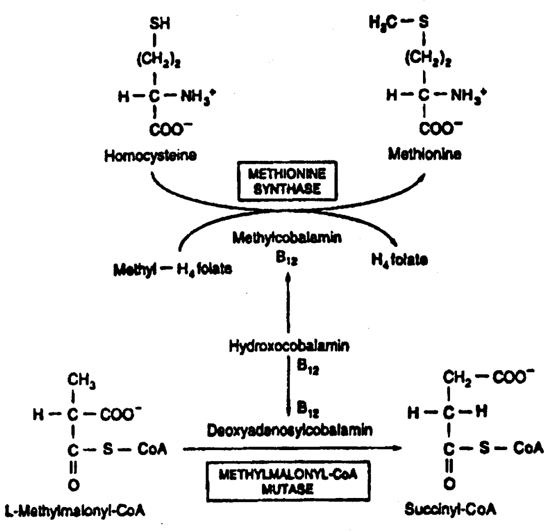
Requirements: Adults: 5 µg/day Pregnant and lactating female: 7 µg/day.

Sources:

- The only source of cobalamin in nature is through synthesis by microorganisms in animal intestine. Neither animal nor plants can synthesize it.
- In general it is not present in vegetable foods.
- The only sources of the vitamin are foods of animal origin as liver, kidney, meats, milk and eggs, negligible amounts are provided by intestinal flora.

Absorption:

Vit. B_{12} is absorbed from the ileum depending on a constituent of normal gastric juice designated intermsic factor (glycoprotein secreted by the partial cells of gastric muscoa).



Reactions catalyzed by cobalamin coenzymes in mammalian tissues

Functions:

1. In food, vitamin B_{12} occurs bound to a protein.
2. To be utilized, vitamin B_{12} is first removed from the protein by acid hydrolysis in the stomach and then combines with the intrinsic factor (a glycoprotein secreted by parietal cells of gastric mucosa) which carries it to the ileum for absorption.
3. As the cobalamin intrinsic factor complex crosses the ileal mucosa, the intrinsic factor is released and the vitamin is transferred to the plasma.
4. The two coenzyme forms of cobalarnin are methyl cobalarnin in the cytoplasm and 5'-deoxyadenosyl cobalamin in mitochondria.

5. In man, there are only two biochemical reactions in which B_{12} is known to participate:
   A- Methylation of homocysteine to methionine occurs in the cytoplasm and utilizes methyl cobalamin as coenzyme and N^5-methyl THF as methyl source.
   B- Isomerization of L-methyl malonly CoA to succinyl CoA by the enzyme L-methyl malonyl CoA mutase and the coenzyme 5'-deoxyadenosyl cobalamin, which occurs in mitochondria.
6. Synths of neucles protein and nucleic acid.
7. Changes of ribose to deoxyribose.
8. Essential for metabolism in hemobiotic system.

Deficiency:

B_{12} deficiency leads to pernicious anaemia, which is characterized by:

- Macrocytic megaloblastic anaemia (thought to be due to the effect of B_{12} on folate metabolism)
- Neurologic disorders due to interference with myelin sheath integrity with sensory and motor losses progressive demyelination of nervous tissue).
- Pernicious anaemia which is characterized by:
  1- Atrophy of gastric mucosa.
  2- Hyperchromic macrocytic anaemia.
  3- Nervous lesions.

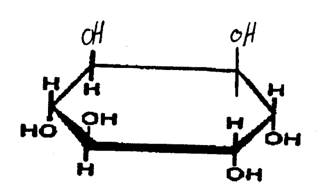
Causes of vitamin B_{12} deficiency:

1- Malabsorption syndrome.
2- Destruction of gastric moucosa or after gastrectomy.
3- Absence of secretion of the intrinsic factor.

10. Inositol:

Chemistry:

- It is a sugar alcohol derived from glucose.
- It is a cyclic compound present mainly in muscles.
- There are 9 isomers of inositol.



Source:

Whole grains, yeast, nuts, and ment.

Function:

It is a lipotropic factor preventing the accumulation of fat in the liver.

Deficiency:

- In mice spectacled eye, alopecia and failure of lactation and growth.
- In chicken exudative diathesis and encephalomalacia.

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11. Choline:

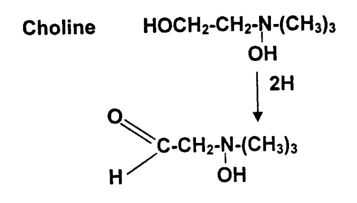
- Quaternary ammonium base.
- Widely distributed in animals and plants.

Was firstly demonstrated as lipotropic factor by best and huntsman in 1932.

Chemistry:

$$HOCH_2\text{-}CH_2\text{-}\underset{\displaystyle OH}{\underset{|}{N}}\text{-}(CH_3)_3$$

- It is regarded as a vitamin.
- It contains 3 methyl groups. So it is a methyl donner.
- In order to give its methyl group, it is changed at first to betaine aldehyde and then to betaine.



Betaine aldehyde

$$HOOC\text{-}CH_2\text{-}\underset{\displaystyle OH}{\underset{|}{N}}\text{-}(CH_3)_3$$

Betaine

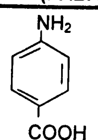
Functions:

- It is a lipotropic factor preventing the accumulation of fat in the liver facilitate formation and secretion of chylomicrons.
- It enters in the formation of lecithins, plasma-logens and sphingomyelins.

Deficiency:

- Rat → hemorrhaye in kidney, eye balls and cirrhosis.
- Chicken → perosis and slipped tendons disease.
- Dog – Rabbits → defect in tibiotarsal joint and cirrhosis.

12- Para-amino-benzoic acid (PABA):



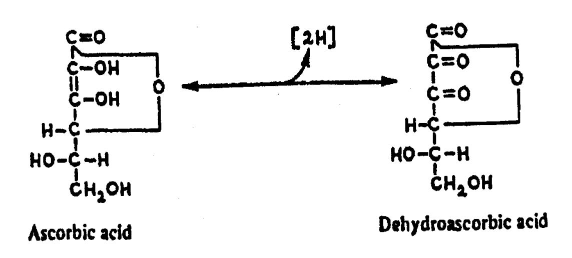
Sources and function:

- It is widely distributed in animal and plant tissues.
- Present in good amounts in yeast.
- It enters in the structure of folic acid.
- It is a growth factor of certain pathogenic bacteria.
- It is antagonized by sulphonamide which has a similar structure.

II. VITAMIN C (L-ASCORBIC ACID):

Chemistry:

- It is γ-lactone of sugar acid.
- It is strong reducing agent.
- It contains enediol group from which removal of hydrogen produces dehydroascorbate. Both forms; ascorbate and dehydroascorbate are physiologically active, found in body fluids and are the major excretory forms.
- Oxalic acid is a catabolic product of ascorbic acid oxidation.
- It can be synthesized from glucose (by uronic acid pathway) in all animals except human and guinea pig.
- It is easily destroyed by cooking. Freezing has no deleterious effect upon this vitamin.
- Very sensitive to oxidation in the presence of copper but not aluminum so foods prepared in copper tans or dishes loss this factor.



Ascorbic acid Dehydroascorbic acid

Requirements: 70-75 mg/day

Sources:

Fresh fruits and vegetables are excellent sources *e.g.*

- Citrus fruits *e.g.* orange and lemon.
- Tomatoes, melons, berries and leaf green vegetables.

Absorption:

- Small intestine.
- Peritoneum.
- S/C tissues.

Distribution: - Parallel to metabolic activities.

Vitamin C requirement:

For the adults the recommended daily allowance is 75 mg for men and 70 mg for women.

N.B.: The most controversial question surrounding vitamin C is its use in megadoses to prevent and cure the common cold. It does not appear to be useful in preventing the common cold, it does appear to moderate its symptoms. It has been suggested that vitamin C is required for normal leucocyte function or for synthesis and release of histamine during stress situations.

Functions:

L-ascorbic acid is the biologically active form, but the stercoisomer, D-ascorbic acid, is not.

1. Ascorbic acid is a reducing agent acting as a cofactor for certain enzymatic reactions for example:
   a. Enzymatic hydroxylation of proline to hydroxy proline in collagen. Thus it is essential for maintairaing the normal intercellular material of cartilage, denentine and bone and the integrity of capillary wall.
   b. It plays a role in tyrosine metabolism.
   c. It is required for the hydroxylation reactions involved in the synthesis of some corticosteroids.
2. Ascorbic acid has other important properties as a reducing agent which appear to be non enzymatic, for example:
   a. It aids in the absorption of iron by reducing it to the ferrous state in the stomach.

b. It enhances the utilization of folic acid by aiding the conversion of folate to tetrahydofolate.

3. Ascorbic acid appears to be a biologically important antioxidant, reducing the risk of cancer when present in adequate amounts in the diet.

Deficiency:

- The normal stores of vitamin C in the body can'nt be depleted thus 3-4 months are required for its deficiency.
- Symptoms of mild vitamin C deficiency include easy bruising and the formation of petechiae (small, pin point hemorrhage in the skin) due to increased capillary fragility.
- Extreme deficiency results in a disease termed scurvy which is characterized by:
    1. Looseness of teeth, inflammation of gums (gingivitis) and bleeding from gums.
    2. Delayed wound healing.
    3. Osteoporosis and easy fractunbility of bones.
    4. Hemorrhage (multiple subcutaneous hemorrhage).
    5. Anaemia (iron deficiency anaemia results from hemorrhage coupled with defects in both iron absorption and folate metabolism).
    6. Increased susceptibility to infections.

B) Fat Soluble Vitamins

- Fat soluble vitamins include A, D, E and K.
- They are non polar, hydrophobic molecules.
- All of them are isoprene derivatives.
- Lipid soluble vitamins require normal fat absorption to be absorbed.
- Once absorbed they are transported to the liver in chylomicrons.
- They are stored either in the liver (A, D and K) or in adipose tissue (E) for varying periods of time.
- In blood they are transported by lipoproteins or specific binding proteins.
- They are not excreted in urine, but are excreted in feces.
- Toxicity occurs from overdosage because of the body's ability to store excess *fat* soluble vitamins (especially A and D).
- No specific coenzyme function bas yet been found for any of the m& soluble vitamins.

1. VITAMIN A:

Isoprene unit

Retinol (vitamin A)

β-Ionone ring α-Carotene α-Ionone ring

β-Ionone ring β-Carotene β-Ionone ring

β-Ionone ring γ-Carotene γ-Ionone ring or Pseudoionone

β-Ionone ring Cryptoxanthine hydroxy β-Ionone ring

Vitamin A is formed from β-ionone ring connected to 9 carbon atoms, the last one is a primary alcoholic group.

Vitamin A_1

Chemistry:

- The three biologically active forms of vitamin A are: retinol, retinal (retinaldehyde) and retinoic acid.
- Retinol is a complex 1ry alcohol of 20 carbon, arranged as a six membered carbocyclic ring and an eleven carbon side chain.
- Vitamin A is derived from the provitamin carotenes.
- The main known carotenes are α, β, and γ-carotenes.
- Synthesis of vitamin A from β-carotene by carotenase (dioxygenase) enzyme occurs in the intestinal mucosa and the liver.

The two common forms of vitamin A are: A_1 in mammalian tissues and marine fishes and A_2 (dehydro A_1) in fresh water fishes.

Requirements: 1 mg/day

Sources:

- Carotenes (pro-vitamin A): dark green and yellow vegetables and fruits are good sources *e.g.* carrots, sweet potatoes, spinach, apricots and green leafy vegetables.
- Preformed vitamin A is supplied by foods of animal origin *e.g.* liver, egg yolk, butter, whole milk and fish liver oil.

Absorption and storage:

- Vitamin A and carotenes are absorbed in the upper pans of small intestine. β-carotenes are convened to vitamin A in liver.
- Vitamin A is stored in liver. The liver synthesize retinol binding protein for transport of vitamin A in blood.

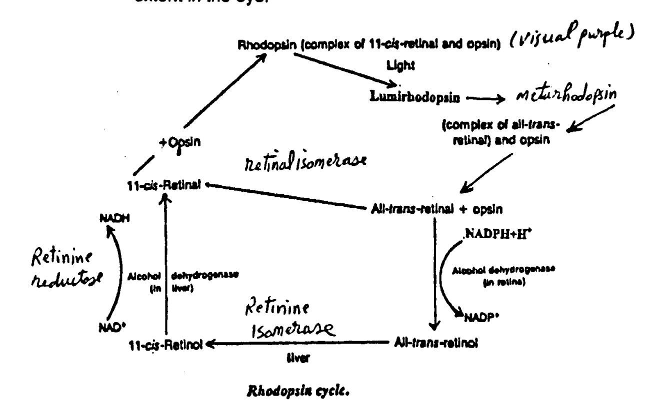
Functions and physiological role:

1. Role of vitamin A as anticancer agent: Vitamin A (retinol and retinoic acid) plays an important role as antioxidant, and might be expected to reduce the risk of cancers initiated by free

radicals and other strong oxidants *e.g.* lung cancer in heavy smokers.

2. Role in cell growth and differentiation:
   - Both retinol and retinoic acid in the target cells are translocated to the nucleus to combine with the DNA.
   - The combined retinol and retinoic acid will stimulate the synthesis of specific mRNA by the nuclear DNA.
   - In the cytoplasm that mRNA stimulate the synthesis of specific proteins by the ribosomes. That proteins will be involved in regulation of cell growth and differentiation.
3. Role in Epithelial Tissue:
   - Vitanrin A (retinol and retinoic acid) is essential for health and integrity of the epithelial tissues especially the secretory epithelium.
   - Retinol can be converted in the body to retinyl phosphate. Retinyl phosphate appears to serve as a glycosyl donor in the synthesis of some mucopoly-saccharide and glycoprotein components of the mucus secreted by the secretory epithelial tissues. Thus, retinol keep the surface of these cells moist.
   - Retinal also inhibits the synthesis and deposition of keratin by epithellal cells, preventing the development of fissures and infection in skin and other epithelial tissues.
4. Role in Vision:
   - Δ^{11}-cis retinal is a component of the visual pigment, rhodopsin, of the retinal rod cells.
   - The retinal rod cells are adapted for vision in low light intensities (night vision).
   - Rhodopsin; the light absorbing conjugated protein, is formed in the retinal rods by binding of the retinal protein; opsin to 11-cis-retinal.

- This synthetic process of rhodopsin proceeds during dark.
- When rhodopsin is exposed to light, the bound 11-cis-retinal is transformed into all trans retinal (change in configuration), this reaction is purely photochemical; it is non enzymatic.
- A series of molecular changes is followed, ending in the dissociation of the bleached rhodopsin to yield free opsin and all trarisretinal.
- These biochemical changes act as a trigger setting off the nerve impulse which will be transmitted through the optic nerve to the center of vision, allowing light to be perceived by the brain.
- Rhodopsin must be reconstituted for continued vision in dim light. This requires isomerization of all-trans retinal back to 11-cis retinal isomerization occurs in the liver and to some extent in the eye.



Rhodopsin cycle.

Deficiency:

Mild deficiency is characterized by:

- Night blindness: impaired vision in dim light is one of the earliest signs of vitamin A deficiency. Follicular hyperkeratosis (rough, scaly and dry skin with keralinization of hair follicles).
- Anaemia: iron deficiency anaemia, but in the presence of adequate iron intake, caused by impaired mobilization of iron from the liver (retinal and/or retinoic acid are required for the synthesis of transferrin).
- Increased susciptibility to infection and cancer are early symptoms of vitamin A deficiency.

Severe Vitamin A Deficiency:

- Xerophthalmia (dry eye due to keratinization of the epithelium of lacrimal glands and loss of tears).
- Keratomalacia (keratinization of the cornea).
- Permanent loss of vision, in the final stages due to infection and hemorrhage.

In animals:

Calf: → Blindness due to constriction of optic nerve due to narrowing of the optic canal.

Dog: → Deafness owing to an injury in auditary nerve.

Poultry: → Sinus troubles.

- ruffeled plumage.
- Staggring gate.

Cattle: → Coubious lacrimation due to keratitis.

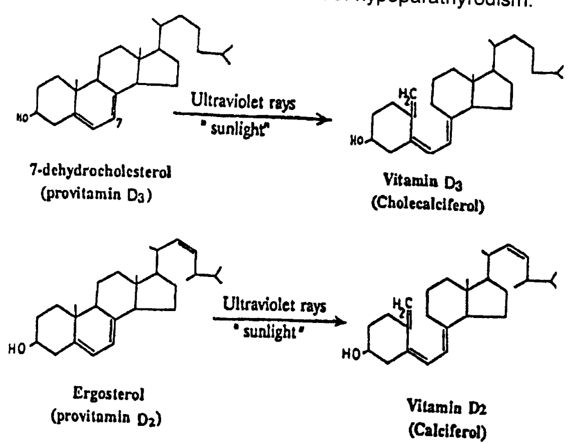
Toxicity (Hypervitaminosis A):

- Large doses over a period of months or years as in treatment of acne can be toxic, since it accumulates in liver.
- Toxic symptoms include: bone pain, scaly dermatitis, nausea, diarrhea, and enlargement of liver and spleen.

2. VITAMIN D:

Chemistry:

- D vitamins are a group of sterol compounds with anti-rickatic activity.
- Vitamin D_2 (ergocalciferol) is generated from ergosterol (provitmnin D_2) by ultraviolet irradiation.
- Vitamin D_3 (cholecalciferol) is derived from 74ehyro cholesterol (pro-vitamin D_3) by irradiation.
- Vitamin D_2 and D_3 are equal biological potency and are metabolized identically.
- Resist oxidation and heat.
- Form esters with fatty acids.
- The irradiation of ergosterol to tachysterolr can be reduced to dihydrotachysterol which exerts powerful hypercalcaemia – stimulate PTH used in treatment of hypoparathyrodism.



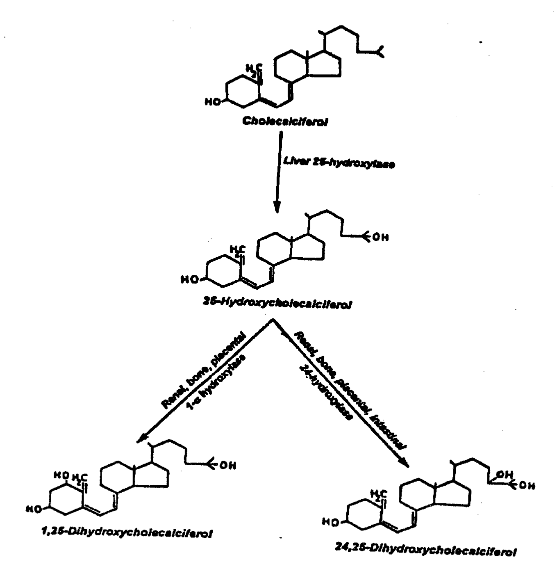
Requirements: 10 μg (400 IU)/day

Sources:

- Cholecalciferol is produced in the skin by U.V. irrdiation of 7-dehydrocholesterol (the cheapest source of vitamin D).
- Preformed vitamin D from fish liver oils, flesh of oily fish, egg yolk and liver.
- Ergosterol in yeast – ergot – snails – egg – milk.

Absorption and Activation:

- It is absorbed through the proximal Small intestine.
- Bound to specific globulin transport it through blood to liver.
- Ergosterol is poorly absorbed but irradiation increased it.
- Bile salts increase the absorption.
- Activation of vitamin D starts in liver cells, where it is hydorxylated on the 25 position by specific microsomal enzyme.
- 25-hydroxy D can be further hydroxylated in kidney by a mitochondrial enzyme to produce 1 α-25 dihyroxy D (calcitriol) which is the metabolically active form of the vitamin.
- Parathyroid hormone (PTH) is essential for the hydroxylaion step in kidney.
- 24, 25-dihydroxy-D is another active form isolated. However, it is less active than 1, 25 dihydroxy D. Although the levels of the two active forms are reciprocally related, they are nearly equal in normocalcemia.



Functions:

- Vitamin D acts as a regulator of the metabolism of calcium and phsophorus, by promoting the transport of calcium and probably secondarily phosphate into the blood stream from intestinal lumen, bones and renal tubules (target organs).
- The mechanism of action of 1, 25 dihyroxy D:
  - **In the intestine:** it induces the synthesis of specific mRNA responsible for synthesis of intestinal calcium binding protein. Thus it increases the absorption of calcium and phosphorus from the intestine.
  - **In the bone:** 1, 25 dihydroxy D and PTH promote bone resorption (mobilization of calcium from bone).

- **In the kidney:** 1, 25 dihydroxy D enhances reabsorption of filtered tubular phosphate, but PTH inhibits both reabsorption of filtered tubular phosphate and excretion of calcium in the kidney.
  - Incorporate phosphorus into phospholipids of intestinal mucosa.
  - Active phytase enzyme inhibiting the action of phytic acid to produce insoluble Ca-phytate.
  - Reduce oxidation of citric acid so increasing it in bone, blood and kidney but not in liver as well as its excretion.

Deficiency:

The deficiency due to:

- Diseases causing fat mal-absorption.
- Severe liver and kidney diseases.
- Drugs interfered with its metabolism as anti-convulsion drugs which inhibit the 25-hydroxylation in liver.

The major biochemical abnormalities of vitamin D deficiency are hypocalcemia and hypophosphatemia, while the most common symptoms are:

1. Rickets in young children: continued formation of osteoid matrix which is improperly mineralized resulting in soft, pliable bones with various deformities as increased bone curvature and enlarged function between bone and cartilage.
2. Osteomalacia: in adults demineralization of preexisting bone takes place, causing the bone to become softer and more susceptible to fracture.
3. In old animals soft bones osteomalacia → diverse deformity.
4. In poultry – soft and rubbery beaks and bowed legs.

Toxicity (Hypervitaminosis D):

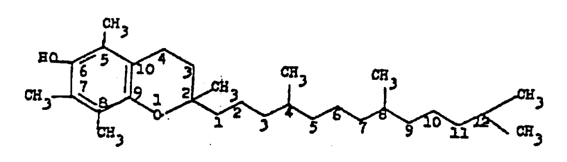
Toxic doses of vitamin D (10-100 dines the daily allowence) lead to enhanced calcium absorption and bone resorption (similar to that seen in vitamin D deficiency) causing hypercalcemia and hypercalcuria which predisposes to formation of renal stones.

3. VITAMIN E (TOCOPHEROLS):

- Rats grown exclusively on cow's milk are in capable of bearing youngs.
- The factor in plant oil [wheat germ oil] restores fertility in male and female rats.
- Greek → Tokos = child birth.
  Phero = to bear
  Ol = an alcohol.

Chemistry:

- Tocopherols (vitamin E) we organic compounds, all have 6-hydroxy chromane ring (tocol) and isoprenoid side chain contairiffig 16-carbons.
- There are 7 naturally occurring tocopherols that differ in the number and site of methyl groups attached to the chromane ring.
- α-tocopherol (5, 7, 8-trirnethyl tocol) is the most active and abundant one.



5,7,8 trimethyl tocol (α-tocopherol)

5,8 dimethyl tocol (β-tocopherol)

7,8 dimethyl tocol (γ-tocopherol)

8-methyl tocol (δ-tocopherol)

Requirements: 8-10 mg/day

Sources:

- Cotton seed oil, corn oil, peanut oil and wheat germ oil are good sources which is the non-saponifiable fraction.
- Green lettuce leaves also have a high content and nearly all green leaved plants have some vitamin E.
- Other good sources include: eggs, muscle meat, liver and fish.

Absorption:

Vitamin E is absorbed through the small intestine, transponed in chylomicrons to the liver and delivered in lipoproteins to periphral tissues (cellular and subcellular membranes and fat deposits).

Functions:

1. Tocopherols have antioxidant activity i.e. they react very readily with molecular oxygen and free radicals, (free radicale is an

atom or molecule that has one or more unpaired electrons, its tendency to acquire an electrone from other substance makes it highly reactive) thus prevent autoxidation of polyunsaturated fatty acids in tissues (cellular and subcellular membranes) by molecular oxygen and free radicals (if oxygen attacks the polyunsaturated fatty acids, fatty acid peroxides will result).

2. The individual molecule in univalent reduction is highly reactive and potentially damages the tissues it may be:
   - Super oxide.
   - Hydrogen peroxide.
   - Hydroxy free radicals (toxic but short lived).
3. During reperfusion injury of ischemic organs xanthin oxidase generate superoxides.
4. Stimulated neutrophils produced superoxides to distruct Bacteria.
5. The antioxident action of vit. E is effective at high O_2 concentration as in RBC_S, alveoli and retina.
6. Vitamin E appears to play a role in cellular respiration either by stabilizing coenzyme Q or by helping transfer of electrons to coenzyme Q.
7. May play a role in keeping stability of erythrocytes.
8. In various animal (but not in humans) it is essential for normal spermatogenesis and continuation of pregnancy.

Deficiency:

Deficiency due to:

- Exposure to high oxygen.
- Mineral oil administration.
- Cholestasis in hepatic diseases.
- A beta lipoproteinemia.
- Intstinal resection.

a) In humans, maturation arrest anaemia and hemolysis due to increased fragility of the red blood cell membrane (presumably due to peroxidation of membrane components). Neurological symptoms have been reported following prolonged vitamin E deficiency.
b) In calf – white muscle disease (muscle dystrophy)
c) In lamb – stiff lamb disease.
d) Rat reproductive failure.
e) Chicken exudative diathesis.

4. VITAMIN K: (Anti-hemorrhagic factor)

- Coagulation vitamin.
- Chick fed synthetic rations develop a hermorrhagic condition characterized by prolonged blood-clotting time.

Chemistry:

- Natural and synthetic vitamin K possess 1, 4-naphthoquinone ring.
- The naturally occurring vitamin K include:
- Vitamin K_1 (phytonadione): 2-methyl-3phytyl-l, 4 naphthoquinone is found in plants.
- Vitamin K_2 (menaquinone): 2-methyl-3 difarnesyl-. 1, 4-napbthoquiflone is synthesized by bacteria in large intestine.
- Synthetically produced vitamin K_3 (menadione) is 2-methyl-1 ,4-napht-hoquinone.
- The naturally occurring group are fat soluble whereas menadione is water soluble.

Vitamin K_1 or phylloquinone

Vitamin K_2 or Farnoquinone

Menadione or, Vitamin K_3

Phthiocol

Requirements: 70-140 µg/day.

Sources:

- Vitamin K_1 is abundant in vegetable oils and green leafy vegetables *e.g.* Spinach, also in cabbage, cauliflower and peas.
- Vitamin K_2 is synthesized by intestinal flora and is found in animal tissues. Putrefied fish meal is a rich source.
- Good sources of vitamin K include tomatoes, cheese, egg yolk. and liver.
- Postnatal period the intestinal flora produced insufficient amount of vitamin K – since the intestine is sterile at birth.
- The quantity supplied by mother is not enough until the microflora become active.
- Prevention by vitamin K administration to mother before parturition.

Functions:

- Vitamin K is required for the conversion of several blood cloning factors (II, VII, IX and X) to the active state.
- All these factors are proteins synthesized in the liver in an inactive precursor form.

- Dicumarol, a naturally occurring anticoagulant, may inhibit the reductase which converts the epoxide back to the active vitamin. An important therapeutic use of vitamin K is as antidote to the dicumarol anticoagulant drugs. It is given either orally; K_1 or parenterally as water soluble vitamin K analogs; menadiol sodium diphosphate.
- The mechanism of vitamin K-dependent activation for prothrombin:
  - Prothrombin is synthesized in the liver in an inactive precursor form called preprothrombin.
  - Conversion of prothrombin precursor to active prothrombin requires a vitamin K-dependent carboxylation of specific glutamic acid residues to 7-carboxyglutamic acid.

COO^-–CH_2–CH_2–(–C(=O)–CH–NH–) $\xrightarrow[\text{VITAMIN K}]{CO_2}$ ^-OOC COO^- >CH–CH_2–(–C(=O)–CH–NH–)

  - The γ-carboxygulatmic acid residues are good chelators and allow prothrombin (active) to bind (chelate) calcium.
  - The prothrombin Ca complex in turn binds to the phospholipid membrane, where proteolytic conversion to thrombin can occur.

Ca (chelated by two O=C–O groups) – CH – CH_2 – HO–C(=O)–CH–NH–H

γ-carboxy glutamic chelates Calcium

- In chicken bone all carboxyglutamate is present in small acidic protein readily extracted by solutions used to demineralize bone this protein named osteocalcin [4-carboxy glutamate + 57 amino acids]
- In kidney another glutamate containing protein occurred in the organic matrix of calcium containing renal stones and in urine.

The chelation of calcium ion by the γ-carboxy glutamyl residue in clotting factor proteins.

To summarize:

1. Vitamin K is an essential cofactor for the carboxylase enzyme that forms γ-carboxygluatinic acid residues from the glutarnic acid residues in to specific protein molecules.
2. Apart from the clotting process vitamin K probably acts as a cofactor necessary for oxidative phosphorylation (coenzyrne Q an essential component of oxidative phosphorylation, is similar to vitamin K in structure).

Deficiency:

- It is manifested by easy bruising and remarkable tendency to bleed profusely from minor wounds.
- Deficiency is diagnosed by prolonged blood coagulation time.
- In poultry: → - Prolonged clotting time.
  - Hemorrhage in breast muscles, legs and wings.
- In ruminants:→Spoiled sweet clover forage contains dicumarol which compete with vitamin K led to bleeding throughout animal body called sweet clover poisoning or bleeding disease.

DETERMINATION OF FAT SOLUBLE VITAMINS

Vit. A: → Vit. A + Antimony trichloride in anhydrous solvents → blue colour.
→ Biological → acceleration of growth of rats.

Vit. D: → Vit. D preparations induced healing of rickets in rats.
→ Chemical → as cholesterol.

Vit. E: → Vit. E + $FeCl_3$ → red colour.
→ Prevent sterility in female rats.

Vit. K: → plasma prothrombin time.

TOXICITY OF FAT SOLUBLE VITAMINS

Vit. A: - High dose inhibit thyroxin.
- Vomiting – headach – white area around mouth.
- Carotenimia → yellow colouration of skin.
  → High dietary carotein.
  → Normal colour of conjunction and urine.
  → Scaly dermatitis hepatomegally.

Vit. D: → hyper calcoemia = calification of kidney – lung – gastric muscosa.
→ Hypercalcuria = renal stons = bone resorption = demoralization.

Vit. E: → The least toxic of fat soluble vitamin.
→ Malaise – easly fatigability.

Vit. K: → hepatomsaly.

MINERALS
OR
ELECTROLYTES



MINERALS

- The minerals, form a small portion of the total body weight, are nonetheless of great importance in tile vital economy.
- Most of the minerals are essential components of an adequate diet.
- The principal sources of the mineral elements are fruits, vegetables and cereals.
- Certain foods are particularly outstanding for their contribution of particular minerals *e.g.*₁ milk products which are depended on to supply the majority of the calcium and phosphorous in the diet.
- The mineral elements present in the animal body are classified into three groups:

1. Major components of body moleculars:

These include carbon, hydrogen, oxygen, nitrogen and sulpher.

2. Principal elements (macronutrients): (major e1ements)

- These includes 7 essential elements which are: calcium, magnesium, sodium, potassium, phosphorous, and chlorine.
- They constitute 60-80% of all the inorganic material in the body.
- They required in amount greater than 100 mg/day.

3. Trace elements (micronutrients): (microelements)

- These are elements that occur in living tissues in small amounts, furthermore their daily requirement is in trace amounts (in μgs or mgs).
- They required in amount less than 100 mg/day.
- Some classify trace elements into essential , possibly essential and nonessential-according to their dietary requirements in higher animals.

- The essential trace elements or micronutrients include: iron, iodine, copper, cobalt, chromium, manganese, molybdenum, selenium and fluorine.

4. Non essential elements:

- These are present in diet but have no essential function in the body. This group includes arsenic, cadmium, nickel, silicon, tin and vanadium.

5. Toxic elements:

- This group contains lead, and mercury.

N.B.:

1. Although in respect to their amounts the mineral elements are relatively minor components of the tissues, they are essential to many vital processes *e.g.* blood calcium exerts an important role in blood clotting and in neuromuscular irritability. despite the relative low content of this element in extracellular fluid.
2. The balance (*i.e.* the ratio of one to another) of the ions in the tissues if often of physiological importance *e.g.*:
    - Normal ossification demands a proper ratio of calcium to phosphorus.
    - Normal activity of muscle requires a normal ratio between potassium and calcium.
3. In. discussing the metabolism of a mineral the following points must be covered: sources, requirements, absorption, excretion and effects of alterations in their blood level (*i.e.* their deficiency or their overdosage).

CALCIUM

Calcium is the major cation present in the body Almost all of it is in the bones and the teeth. the very small (1⁰/o) not in the skeletal structures is in the body fluids and is in part ionized. The

total body content of an average 70 kg adult is about 1200 g. 99% of them in the skeleton.

Functions of calcium

1. Enters in the structure of bones and teeth.
2. Maintenance of normal excitability of heart muscles arid nerves.
3. Essential for blood cloning, and milk curdling.
4. Maintenance of integrity of capillary walls and decreases their permeability.
5. Activation of certain enzymes as pancreatic lipase and glycogen phosphorylase.
6. A normal blood calcium is essential for release of almost all hormones. The only two hormones that are released in response to low blood calcium are: glucagon and parathyroid hormone (PTH).
7. May play a role in mediating the action of some hormones, in this respect they act as a third messenger, (cyclic AMP is the second messenger and the hormone is the first messenger).
8. It involves in the regulation and secretion of insulin.

Sources of calcium:

1. The richest sources of calcium are milk and cheese.
2. Most other foods contribute smaller amounts *e.g.* egg yolk, beans, lentiles, nut, figs, cabbages, cauliflower and turnip.

Requirements

- Children : 0.8 – l.2 g/day
- Adults: 0.8 g/day.
- Pregnancy and lactation 1.2 g/day.

Absorption of calcium :

- This occurs by an active transport mechanism in the upper part of small intestine.

Factors Affecting Absorption

1- pH of duedenum:

- An acidic duedenal pH is essential for normal absorption as calcium is insoluble and non-ionized in alkaline pH.
- An acidic pH is provided by the acid chyme, on its arrival for stomach.

2- Phosphate in diet:

- Increasing the amount of phosphate in diet precipitates calcium and decreases its absorption.
- The Ca : P ratio in diet must be 1 : 2 or 1:1 for optimum absorption, this is the ratio present in human milk. (<u>animal milk contains higher phosphate content and this may produce hypocalcaemia after absorption of phosphate lending to tetany and convulsions in infants</u>).

3- Forms of calcium in Diet:

- The organic forms *i.e.* calcium gluconate, lactate or citrate are soluble and readily absorbed.
- The inorganic forms *e.g.* calcium carbonate and calcium phosphate are insoluble and poorly absorbed.

4- Other constituents of diet

- Phytic acid (in cereal grains) interfere with calcium absorption by forming the insoluble calcium phytate.
- Oxalates in food (*e.g.* in spinach) has a similar effect by forming calcium oxalate
- High protein diet increases calcium absorption.

5- Vitamin D

- This is essential for and promotes absorption of calcium from the intestine (see its role later on).

6- Parathyroid hormones: essential for formation of the active form of vitamin D (1, 25 dibydroxy cholecalciferol or calcitriol).

Excretion of Calcium: This occurs in: milk, urine and stools.

Blood Calcium

- The normal plasma level = 9-11 mg% (average 10 mg%).
- The erythrocytes not contain calcium.
- The plasma calcium exists in 2 forms:
  1- Non-diffusible (45%):
    - This form of calcium is bound to plasma proteins maily albumin.
    - It is physiologically mactive.

  2- Diffusible (55%):

  This form of calcium may be:

  A- Ionizable (50%).
    - This mostly in the form of chloride salt.
    - It is the only physiologically active.
    - If it decreases tetany occure.

  B- non-Ionizable (5%):
    - This mostly in the form of citrate salt.
    - It is physiologically inactive.

Factors Affecting Blood Calcium:

1. **Parathyroid hormone (PTH)** which is a **hypercalcaemic** hormone *i.e.* increases blood calcium by
   - Increased mobilization of calcium from bones.
   - Keeps Ca X P solubility product constant (10 x 5 = 50).
   - Increased calcium absorption from intestine (by activation of vitamin D).
2. **Calcitonin** which is a **hypocalcaemic** hormone that decreases blood calcium by increasing calcium deposition in bones and also it inhibits the osteoclastic activity. On the kidney it increases calcium excretion.

3. **Ca X P** solubility product which must be kept constant (at 50 mg) i.e. if phosphates are increased in blood, calcium level will decrease, and vice versa.
4. **Vitamin D** (active from) which increases calcium absorption from intestine. The active from of vitamin D *i.e.* 1.25 dihydroxy cholecalciferol enters the intestinal mucosal cells (after being synthesized in kidney) where it acts on the DNA of the mucosal cell nuclei to stimulate the synthesis of a specific messenger RNA (mRNA). This specific messenger RNA will be transported to the ribosomes in the cytoplasm of the mucosal cells to stimulate the synthesis of a calcium-binding protein that it will trigger the transport of calcium, from the intestinal lumen to the blood.
5. **Blood pH** : calcium is soluble and ionized at the normal blood pH (7.4). On the other hand, alkalosis decreases ionization of calcium.

N.B.: The hormones that regulate blood calcium (calcitropic hormones) are:

1. Parathyroid hormone.
2. Calcitonin.
3. 1, 25 dihydroxy cholecalciferol (active vitamin D or calcitriol).

Notes on parathyroid Hormone (PTH) Action:

1. Parathyroid hormone produces proliferation of osteolytic cells in bone (mainly osteoclasts), which produce bone resorption involving bone matrix and mineral crysia. and leading to a net mobilization of calcium from bone.
2. The stimulus for parathyroid hormone release is the decreases in serum calcium level (hypocalcaemia).
3. The action of parathyroid hormone is possibly mediated through cyclic AMP.

ALTERATIONS IN SERUM CALCIUM LEVELS

Hypercalcaemia, which can be produced by:

1. Primary hyperparathyroidism:

- This results from increased secretion of PTH than what is needed.
- This usually occurs due to single adenoma in the gland (85-90%) and less frequently due to hyperplasia of all four parathyroid glands (5-10%).

2. Secondary hyper parathyroidism:

- This is a state characterized by an increased release of PTH to compensate for a decreased serum ionized calcium i.e. secondary to the decrease in calcium level.
- The decreased serum calcium level usually results from renal failure, osteomalacia, steatorrhea,, or post-gasteroctomy malabsorption.

3. Hypercalcaemia of malignancy:

- The most common from of this type occurs due to direct infiltration of bone by malignant tissue which produces oesteolysis and increased mobilization of calcium to blood.
- Other forms include hypercalcaemia associating breast cancer and multiple myeloma where parathyroid hormone-like peptides are possibly released from the tumors to produce hypercalcaemia.

4. Milk-alkali syndrome:

- This occurs in patients who take antiacids and drink milk for peptic ulcer disease.
- In such condition there is an increased calcium absorption (from the ingested milky which is not balanced by increased renal excretion.

Hypocalcaemia and Tetany:

Tetany is a condition characterized by increased neuromuscular irritability (excitability) due to a decrease in ionized calcium in blood that may result form: (causes of hypocalcaemia):

3- Hypoparathyroidism.
4- Alkalosis (which decreases serum ionized calcium).
5- Decreased dietary intake or poor absorption from intestine.
6- Kidney disease (nephritis) that increases renal excretion of calcium and inhibits vitamin D activation in kidney.

PHOSPHORUS

- Phosphorus is found in every cell of the body.
- It presents in 2 forms:-
  - A- Inorganic form *e.g.* Na, K and Mg salts.
  - B- Organic form esters of phosphoric acid with organic compounds *e.g.* G.6.p and phospholipids.
- 70% of food phosphates are absorbed.
- Aluminum hydroxide (ant-acid) inhibit absorption due to the formation of aluminum phosphate.
- Total body phosphorus averages 0.80 kg/70 kg.
- 80% of the total body P is in the skeleton.
- Blood phosphorus:-
  - A- Erythrocytes:
    - 1- Most of the phosphorus in the form of organic phosphates.
    - 2- Very little is inorganic salts of potassium phosphate.
  - B- Plasma:
    - 1- Part of plasma P is organic in the form of phosphlipids.
    - 2- The inorganic phosphates is of sodium phosphate type.

- Renal function effect plasma inorganic phosphate which increased in renal failure due to defect in excretion.
- Normal serum inorganic phosphate level is 2.5-4.5 mg% (0.9-1.5 mEq/L).

Forms and Function:

1. Enters in bone and teeth formation.
2. Enters in structure of phospholipids.
3. Enters in structure of nucleic acid (DNA and RNA).
4. Enters in structure of coenzymes as NAD and FAD.
5. Present as phosphorylated intermediates of carbohydrates and lipids *e.g.* glucose-6-phosphate and glycerol-3-phosphate.
6. Enters in structure of high energy phosphate containing compounds *e.g.* ATP, GDTP, CTP, ...etc.
7. Enters in structure of cyclic AMP and cyclic GMP.
8. Enters in the formation of buffers.

Sources, Absorption and Excretion:

- The same as that for calcium.

High Energy Phosphate Compounds: These are:

1. ATP and its relatives *e.g.* GTP and CTP.
2. Creatine phosphate.
3. Carboxyl phosphate *e.g.* the phosphate bond at C-I in 1, 3 diphosplioglyceric acid.
4. Enol phosphate *e.g.* phosphoenol pyruvic.
5. Carbamoyl phosphate (urea cycle).

<u>N.B.:</u> High energy compounds include:

1- High energy phosphate.

2- High energy sulfate (see sulfur).

MAGNESIUM

- Magnesium is one of the principal elements (macroelements).
- The total body content of magnesium is about 21 gm.
- 70% is combined with calcium and phosphorous in bone and the remainder is present in soft tissues and body fluids

Sources:

- Excellent sources are cocoa derivatives, soybeans and various nuts.
- Other sources include whole grains, raw dried beans, and peas.
- The major source is the chlorophyll of plants.

Distribution:

- 70% in the skeleton.
- 30% in the other tissues and body fluids.
- It mostly intracellular.
- Its concentration in muscle cells is about 10 times in plasma.

Blood magnesium:

- Plasma mg normally 2.40±0.60 mg/dl.
- 80% of them is diffusible.
- 20% is non-diffusible bound to plasma proteins.
- Erythrocyte mg is 2-3 times of plasma.

Factors affecting plasma magnesium:

1. *Aldosterone:*
   - Decrease plasma mg by increasing its urinary excretion.
2. *Parathyroid hormone:*
   - Increase plasma mg by increasing its mobilization.
3. *Kidney function:*
   - Hypermagnesaemia in renal failure due to defect in excretion.

Requirements:

- 350 mg/day for men and 300 mg/day for women.

Absorption:

- This occurs in upper small intestine – Normally about 40% of the ingested.

Factors affected mg absorption:

1. Amount of Mg in diet.
2. Amount of Ca in diet due to Ca competes with mg absorption.
3. Vit. D.
4. Parathyroid hormone.
5. Solubility of Mg as high and excess phosphate, phytic acid and unabsorbed fatty acids effect its solubility and absorbabability.

Excretion:

- In urine and milk. Aldosterone increases the renal excretion of magnesium as it dot also with potassium.

Function:

1. Activation of many enzymes as kinases, adenylate cyclase, gunnylate cyclase, phosphorylase and some decroxylase.
2. Acts to depress neuromuscular rind central transmission.
3. Inter in the formation of skeleton.
4. Important for normal contraction of muscles.
   - Hypermagnesamia leads to muscle weakness and paralysis this action antagonized with calcium.
5. It decreases neuromuscular excitability.
   - Hypomagnesoemia leads to tetany which can'nt be treated with calcium.

Alterations in serum Mg^{++}:

1. Magnesium deficiency, this is charactedzed by confusion, neuromuscular irritability, and seizures similar to the symptoms of hypocalcaemia.
2. Hypermagnesemia may produce hypotension, loss of tendon reflexes, stupor, and coma, and it rarely occurs following increased intake of magnesium in laxatives and as magnesium sulfate given in the reatment of eclampsia.

SULFUR

Sources:

- The major sources of sulfur are the sulfur containing amino acids: cysteine, cystine and methionine.

Form of sulfur in body:

1- Sulfur containing amino acids present in structure of tissue proteins. plasma proteins, enzymes and hormones.
 - These include cysteine, cystine and methionine.
 - They may undergo the following metabolic changes.

 A- Oxidation:
  - In the liver partially separates sulpher to thiosulphate S_2O_3 and sulphite SO_3^- both of which oxidized to inorganic sulphate SO_4.
  - Taurine from partial oxidation of cysteine.

 B- Conversion to neutral sulpher compounds as:
  - Proteins specially keratine.
  - Glutathione.
  - Homocysteine.
  - Amino ethyl mercaptan (in Co ~ A).
  - Mercapturic acid (detoxication of Aromatics).
  - Thiocyanate (detoxication of cyanides).
  - Urochrome (a peptide substance in urine)

 C- Excretion in urine-very little of the S-containg amino acids excereted in normal conditions.

2- Sulfur containing vitamins: thiamine (B_1), biotin and lipoic acid.

3- Sulfur containing coenzymes: TPP, lipoic, biocytin, COASH and glutathione.

4- Sulfolipids.

5- Bile salts (sodium and potassium salts of taurocholic acid).

6- Sulfated mucopolysaccharides as heparin and chondroitin

sulfate.

7- Lipoic acid derivatives *e.g.* acetyl lipoate atd succinyl lipoate.
8- COA derivatives *e.g.* active acetate, active succinate...etc.
9- Active sulfate
10- Ergothionine is found in liver, RBC_S and semen.

High energy sulfur containing compounds include:

1- COA derivatives e.g. active acetate, active succinate .....etc.
2- Lipoate derivatives *e.g.* acetyl lipoate and succinyl lipoate.
3- Active methionine (used for transmethylation reactions).
4- Active sulfate (used in detoxication).

Excretion:

A- In urine:

- This is the principle rout of excretion.
- About 1 gm excreted daily in the form of:

1- Inorganic (80%):
   - Na and K sulphate related to protein intake and catabolism.

2- Neutral sulpher compounds (10%):
   - Sulpher containing vitamins and amino acids.
   - Thiocyanates and urochrome.

3- Ethereal sulphate (10%):
   - Include
     - indican (K-indoxyl sulphate)
     - Skatoxle K-sulphate.
     - Phenol sulphate.
     - Steroid hormone sulphate.
   - They relatively constant exception increased intestinal purification.

4- **In addition there are some S-containing compounds as taurine, taurocholic acid and thiosculphate.**

SODIUM

- Sodium (Na^+) is the major cation of the extracellular fluid and is largely associated with chloride and bicarbonate in the regulation of acid-base balance.
- The plasma sodium concentration is about 142 m.Eq/litre (330 mg %).
- The main dietary source of sodium is table salt (NaCl) used in cooking and seasoning. Meats contain more sodium than vegeratbles.
- Requirements: about 5 g/day.
- Absorption readily occurs in ileum, and little is present in stools.
- Excretion occurs in urine and sweat.
- In susceptible individuals, there is a clear relationship between Na^+ intake and diastolic blood pressure.
- Thus the excessive and wasteful intake of NaCl may lead to or aggrevate pre-existing hypertension.
- Sodium intake must be decreased in hypertension. renal failure and some cardiac diseases.

Factors affecting plasma level of Na, K and chloride:

1. Rapid and prolonged transfusion for injected fluids.
2. Acid-base balance.
3. Vomiting and Diarrhia.
   Vomiting short period → alkalosis and hypochloremia
   Prolonged vomiting and diarrhea → hypernatremia, acidosis and hypokalemia.
4. Excessive sweating and diabetes insipidus lead to hypernatremia and hyperchloremia.
5. Renal functions.
   Chronic renal failure leads to hyponatremia without hyperkolemia.

6. Diuretics
   Leads to hyponatremia and hypokalemia.
7. Adrinocortical function:
   a) hyperfunction (cusing disease) → hypokalemia-hypo-Cl leads to alkalosis
      and hyper-Na leads to increase blood volum and pressure.
   b) Hypofunction (Addison disease) → hyper-K and hyper-Cl leads to acidosis and hypo-Na leads to decrease blood volume and pressure.
8. Intravenous injection of glucose.
   Each gram glycogen stored –0.36 mol of K enters the cell leads to hypo-K.

Function of sodium:

A- Regulation of:

1. Osmotic pressure of extracellitlar fluid.
2. Acid-base balance (formation of buffers).
3. Water balance.

B- Plays a significant role in neuromuscular excitation process by preserving the normal irritability of muscles and permeability of cells.

Sodium depletion (hyponatraemia):

The plasma sodium concentration may be decreased in the following disorders:

1. Renal diseases characterized by failure of renal tubular reabsorption mechanisms with an increased loss of Na^+ in urine e.g. sodium losing nephritis.
2. Addison's disease: due to deficiency of aldosterone (aldosterone normally stimulates Na^+ tubular reabsorption in distal tubules).
3. Excessive loss in gastrointestinal fluids as in severe vomiting and diarrhae (the gastrointestinal secretions are rich in Na^+ and K^+).

4. By the excessive action of diuretics that block tubular sodium reabsorptive mechanisms.

Effects of hyponatraemia:

1. Neuromuscular weakness.
2. Lethargy, confusion, coma and death in severe cases.

Sodium intoxication (hypernatraemin):

- Sodium may be retained in high concentration in the body in the following states.

1. Cushing's syndrome: the excessive glucorticoids present in such state will increase sodium reabsorption in distal tubules.
2. Excessive cortisone intake.
3. Hyperaldosteronism.

Effects of hypernatraelnia:

- The major effect is cellular dehydration, and dehydration of brain cells will lead to lethargy, convulsions, coma and even death, if not treated.

POTASSIUM (KALIUM)

- Potassium is the principal cation (K^+) of the intracellular fluid.
- The plasma concentration is about 5 m.Eq/litre (20 mg %).
- The main dietary sources are meats, vegetables and fruits (especially lemons, oranges and banana).
- Absorption readily occurs in upper part of small intestine.
- The daily requirement is about 3-4 g/day.

Function of potassium:

- A high intracellular potassium is required for.

1. Protein synthesis by the ribosomes.
2. Activation of some enzymes as pyruvate kinase and sodium-potassium adenosine triphosphatase (Na^+ + K^+ ATPase, of sodium pump).

3. Maintenance of membrane potential of excitable tissues notably cardiac muscle.
4. Regulates intracellular: acid-base balance, osmotic pressure and water balance.

Potassium depletion (Hypokalaemia):

- This occurs in:

1. Cushing's disease (adrino cortical hyperfunction).
2. Hyperaldosteronism.
3. Excessive steroid intake.
4. Alkalosis: the decreased H^+ concentration in plasma will result in shift of H^+ from intracellular fluid (JCF) into extracellular fluid (ECF), with a reverse shift of K^+ (from plasma into cells) to keep the concentration of intracellular cations. This will result in decreased serum K^+ concentration (hypokalaemia).
5. Dietary deficiency specially in elderly persons who live on tea and toast.
6. Duiretics that increases K^+ loss.
7. Increased losses by vomiting and diarrhea.

Effects of hypokalnemia:

1. Tenderness, weakness and hyporeflexia.
2. Cardiac arrhythmias.
3. Renal tubular damage (in prolonged cases) with polyuria and failure to concentrate urine.

Causes of hyperkalaemin:

1. Excessive intake specially in the presence of impaired renal function.
2. Increased tissue damage (due to the release of the high K^+ content).
3. Addison's disease (due to absence of aldosterone, which normally increase K^+ and H^+ excretion in exchange for the reabsorbed Na^+).

4. Renal failure; due to failure of filtration at glomeruli and/or secretion by tubules.
5. Acidosis: The intracellular shift of H^+ for buffering will lead to extracellular shift of K^+ (from buffering will lend to extracellular shift of K^+ (from within cells into plasma).

Effect of hyperkalaernia:

1. Generalized neuromuscular irritability with hyperreflexia.
2. Myocardial irritability leading to arrhythmia and cardiac arrest.

The sodium-potassium pump:

- The mechanism responsible for the active transport of sodium out of the cell and potassium into the cell is a sodium-potassium pump (simply known as sodium pump).
- The pump is located in the membrane and the energy for pumping is provided by ATP generated during metabolic reactions in the cell
- An enzyme intimately related to the active transport of Na^+ and K^+ across cell membrane has been recently described.
- This enzyme hydrolyses ATP to adenosine disphosphate (ADP), and it is activated by Na^+ and K^+. It is therefore known as the sodium-potassium activated adensine triphosphatase (Na^+ -K^+ ATPase or transport ATPase).
- The enzyme is absolutely dependant upon the presence of Na^+ for its activity, but other ions as lithium (Li^+ can substitute for K^+ to some extent).
- Also the enzyme requires Mg^{++} for its activity and so it is called Mg^{++}-activated ATPase.
- Tissues with high transport activity, including nervous and secretory tissues possess high Na^+-K^+ ATPase activity.
- The transport mechanism is inhibited by ouabian (cardiac glycoside = drug used in treatment of heart failure). Also it is inhibited by metabolic poisons which prevent the formation of ATP.

CHLORIDE

- This is the main anion of extracellular fluid.
- Its concentration in plasma is about 103 m.Eq/litre.
- It is always present associated with sodium (as NaCl) and so its sources are the same as sodium.
- Absorption occurs in the upper part of small intestine.
- Excretion occurs in urine and sweat.
- Requirements 5-15 g/day (in the form of NaCl).

Functions:

A Togerher with sodium it is essential for:

1. Regulation of water balance.
2. Regulation of acid-base balance (by chloride shift).
3. Regulation of osmotic pressure.

B- Essential for production of HCl of gastric juice.

Hypochloremic alkalosis:

- This condition mainly occurs after prolonged vomiting (as in pyloric obstruction) where there is loss of chloride in excess of sodium.
- This leads to a decrease of plasma chloride, with a compensatory increase of plasma bicarbonate and a resultant hypochloremic alkalosis results.
- Other causes for such condition include:

1. Cushing's disease.
2. Excessive intake of ACTH or glucocorticoids.

- In these two later condition there is also hypokalemia.

TRACE ELEMENTS

IRON

- Iron is one of the trace elements.
- The total body iron ranges between 3-5 g and is distribution:
  Iron present in the body in 2 forms:-

A) Functional form (75%):

These are mostly hemoproteins responsible for cellular respiration they include:-

1- Hemoglobin (67%)
- The main form of iron in the body,
- It carries O_2 from lung to tissues.
- Help in carriage of CO_2 inopposite direction.

2- Myoglobin (7.5%)
- A hemoprotein found in muscles.
- Temporary carry oxygen.

3- Respiratory enzymes (0.5%).
- Hemoproteins include cytochromes and cytochrome oxidase (in respiratory chain).
- Catalase and peroxidase important in detoxication of hydrogen peroxide.
- Tryptophan pyrrolase in tryptophane metabolism.
- Flavo-protein enzymes contain non-heme iron as NADH-dehyrogenase and succinate dehydrogenase.

B) Non functional form (25%) (transport and storage of iron):
- Transferrine → transport form in plasma.
- Ferritine → shelf storage form in liver, kidney, bone morry.
- Hemosidrin → when body contain excess iron.

Function of iron:

- Iron is the element of great importance regarding oxygen metabolism.
- Its role in this respect can be classified as follows:
  1. Oxygen carriage by hemoglobin.
  2. Oxygen storage: by myoglobin
  3. Oxygen utilization: by respiratory chain.
- Important for hemopiosis and hematopiosis.

Sources of iron:

1. Organs meat: liver, heart, kidney and spleen.
2. Egg yolk and fish.
3. Plant sources as whole weat, dates, nuts, artichoke, beans and figs.

Requirements:

- 10-20 mg/day for normal adults (only 1-2 mg/day are absorbed which is the actual requirement).
- This is increased during period of increased demand *e.g.* pregnancy, lactation and after blood loss.

Absorption:

1. Iron is present in diet as ferric organic complex. By action of HCl in the stomach ferric ions (Fe^{+++}) are liberated.
2. Ferric ions are reduced to ferrous (only ferrous ions can be absorbed). This reduction is carried out by H^{+} of HCl, SH groups of proteins and by vitamin (C).
3. Ferrous ions (Fe^{++}) are absorbed to be oxidized again inside the intestinal mucosal cells to ferric (Fe^{+++}) ions,. then the ferric ions combine with apoferrition (mucosal cell protein) to form ferritin.
4. Ferritin liberates ferric ions into the plasma and apoferritin is regenerated.

Iron binding by transferring:

The precise mechanics of iron loading onto transferring as it leaves intestinal epithelial cells or reticuloendothelial cells is unknown. The copper-dependent ferroxidase, ceruloplasmin. May play a role. Compelling evidence indicates that the protein is involved in mobilizing tissue iron stores to produce diferric tranferrin. Ferric iron couples to transferring only in the company of an anion (usually carbonate) that serves as a bridging ligand between metal and protein, excluding water from two coordination sites. Without the anion cofactor, iron binding to transferring is negligible. With it, ferric transferring is resistant to all but the most potent chelators. The remaining four coordination sites are provided by the transferring protein-a histidine nitrogen, an aspartic acid carboxylate oxygen, and two tyrosine phenolate oxygens. Available evidence suggests that anion-binding takes place prior to iron-binding. Iron release from transferring involves protonation of the carbonate anion.

Distribution and kinetics of body iron:

Percent of total	Iron (grams)	Compartment
66%	2.7	Hemoglobin
3%	0.2	Myoglobin
0.1	0.008	Heme Enzymes
--	<0.0001	Non-heme Enzymes
30%	1.0	Intracellular Storage (Ferritin)
1%	0.07(?)	Interacellular Labile Iron (Chelatable Iron)
0.1%	0.003	Intercellular Transport (Transferrin)

Mucosal block:

- This is the theory which was previously described to account for the intestinal control of iron absorption.

- It states that the intestinal content of apoferritin is limited, and so once saturated with (to form ferritin), block or inhibition of further iron absorption is carried out.
- However more recently a specific active transport system for iron was described and variation in the activity of this system regulates iron absorption.

Factors regulating iron absorption:

1- Requirements of body:

- An increased rate of erythropoiesis (*e.g.* after hemorrhage increases the need for more absorption of iron.

2- Forms of iron in diet:

- Organic iron e.g. iron citrate, lactate and gluconate and more soluble and readily absorbed.
- Inorganic irons as iron phosphate, carbonate are insoluble and poorly absorbed.

3 Other constituents of diet:

- Vitamin (C) and proteins increase iron absorption.
- Increased phosphate and oxalate precipitate iron and hinders its absorption. Also phytic acid produces the same effect i.e. decreases absorption.

N.B.:

Absorption of iron occurs mainly in the stomach and duedenum. So after gasterectomy (removal of stomach) the patient is liable to iron deficiency anemia.

Blood iron:

1- In red blood cells:

- It contains Hb which contain 3.40 mg of Fe/g.
- There are 15 g of Hb/100 ml blood.
- That amount of Hb = 50 mg Fe/100 ml blood.

2- In plasma:

- Plasma Fe concentration – is 50-150 μg/dl.
- Fe carried on glycoprotein (transferrine).
- Each molecule of transferrine carried 2 atoms of ferric.
- Transferrine synthesized in liver.
- Transferrine may carry up to 250-400 μg of Fe/dl of plasma which is the total iron binding capacity (TIBC).
- This means that on the average only about 30% of TIBC is saturated.
- In iron deficiency anemia plasma Fe decreased and TIBC tends to increases.
- In liver diseases plasma Fe and TIBC are decreased.
- Plasma contain-s only low concentrations of ferritin which is the index of Fe storage.
- Ferritin decreased in iron deficiency anemia and increased in hemosiderosis.

Storage of iron:

Iron is stored as ferritin (mainly) in the following organs and tissues:

1- Liver. 2- Intestine.
3- Spleen. 4- Bone marrow.
5- Kidney. 6- Heart.

Excretion of iron:

- This amounts to 1-2 mg/day mainly in stools but also in sweat, hairs and menstrual blood in females.

Iron differs from most other minerals in that its quantity in the body is controlled by regulation of absorption rather than its excretion.

1- In stool (90-95%):

- Fecal Fe mostly unabsorbed Fe.
- Only few amount excreted in bile after absorption.

Comparison of Iron Losses

	Source of loss	Extra loss	Daily extra loss	Daily total loss
Mean and non-menstruating women	**Desquamation**	-	-	**18 µmol** *(1 mg)*
Menstruation women (Mean value)	**Desquamation + menstruation**	**290 µmol** *(16 mg)/ month*	**9 µmol** *(0.5 mg)*	**27 µmol** *(1.5 mg)*
Pregnancy	**Desquamation + loss to fetus and in placenta**	**7000 µmol** *(380 mg) / 9 months*	**27 µmol** *(1.5 mg)*	**45µmol** *(2.5 mg)*
Male blood donors	**Desquamation + 1 unit blood**	**4500 µmol** *(1 mg) /4 months*	**36 µmol** *(2.0 mg)*	**54 µmol** *(3.0 mg)*



2- In urine and sweat (5-10%):

3- In menstruation and milk (5-10%):

- About 15-30 mg of iron (Hemoglobin) are lost in menstruation per month *i.e.* 0.5-1.0 mg/day.
- Breast feeding accounts for the loss of 0.5-1.0 mg/day.

4- The body is unable to excrete a large load of iron.

N.B.:

After intake of excess amounts of iron the stools are rendered black due to oxidation of iron in colon with excretion of iron oxide (black) in stools which must be differentiate from meleana (gasterointestimal bleeding).

Bronz diabetes: (Hemosiderosis-hemochromatosis).

This is an abnormal condition characterized by uncontrolled iron absorption resulting in increased rate of iron absorption, the absorbed iron will be deposited in the form of hemosidrin in:

1. Subcutaneous tissues → bronz colour.
2. Pancreas → destroy B-cells → diabetes.
3. Brain → mental disturbances
4. serum Fe is elevated – transferring becomes 70-90% saturated similar condition in patients with a plastic or hemolytic anemia who recovered repeated blood transfusion.

N.B.:

1. Iron deficiency results in hypochromic microcytic anemia due to low intake of iron.
2. Iron balance is at equilibrium in normal adults *i.e.* intake = excretion n = 1-2 mg which equals the amount excreted).
3. Hemochromatosis – due to iron overload for long time there are hemosidrive deposits in liver, pancrease skin and joints.

IODINE

- Total body contain 25-50 mg.
- Iodine is one of the trace elements.
- Source vegetable and fish.
- The only known function of iodine is synthesis of the thyroid hormones which proceeds by the following steps:

1. Trapping of inorganic iodide are oxidized by iodine peroxidase enzyme to molecular iodine (elemental or organic iodine), copper is needed as activator for this enzymatic step.

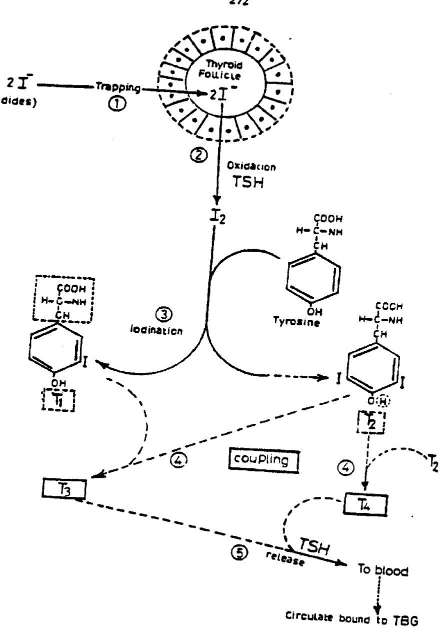
$$\textbf{Iodide} \xrightarrow[\textit{Peroxidase}]{\textit{TSH, } Cu^{++}} \textbf{Iodine}$$

   TSH = thyroid stimulating hormone.

2. Iodination of tyrosine:
   - This is carried out while the tyrosine molecules are attached to thyroglobulin (thyroid protein).
   - The result of iodination is the formation of:
     T_1 = monoiodo-tyrosine.
     T_2 = diiodo-tyrosine.

3. Coupling (conjugation) of:
   $T_1 + T_2 \rightarrow$ forms T_3 = triiodo-thyronine.
   $T_2 + T_2 \rightarrow$ forms T_4 = tetra-iodothyronine = thyroxin $\rightarrow$
   in peptide linkage.
   Both T_3 and T_4 are the thyroid hormones.

4. Release T_3 and T_4 from thyroglobulin contain 115 tyrosin residue into plasma occurs by a proteolytic enzyme and is activated by TSH. The released thyroid hormones are transported bound to plasma proteins known as thyroxine binding globulins (TBG).



Synthesis of thyroid Hormones

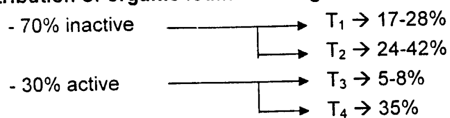
Absorption:

Readily absorbed in skin, lung, intestine converted to inorganic iodide before absorption intestine + stomach

Iodine in the body:

- 25-50 mg.
- 10-15 mg in thyroid.
- 2 mg/g dried thyroid
- 100 : 1 – 10:1 ratio of thyroid iodine.

Distribution of organic iodine in the gland:



N.B.:

1. T_3 is more active, more rapidly acting and more rapidly excreted than T_4.
2. 80 % of thyroid hormones stored in the thyroid gland is in the form of T_4, the remaining 20 % are present as T_3.
3. Plasma free levels of free T_3 is relatively more higher than that of T_4, due to the poor binding of T_3, to plasma proteins.
4. Both T_3 and T_4 are metabolized in the peripheral tissues by demination and decarboxylation to produce.
   * TRIAC : triiodoacetic acid.
   * TeTRAC: tetraiodoacetic acid.
5. Tetrac and Triac possess 25% of activity of T_3 and T_4.
6. Thyroid hormones are conjugated in liver with glucuronic acid and sulfate, and the conjugated products are excreted in bile and urine.

Excretion $\rightarrow$ mainly in urine and sometimes in stool, saliva lungs and milk.

COPPER

- High concentration in liver.
- Copper is one of the trace elements.
- Whole body content is 100-150 mg.
- Although its exact function is not yet understood but copper is known to be:

1. Essential for hemoglobin synthesis (and hence normal erythropoiesis), normal bone
   formation and the maintenance of myelin within the nervous system
2. Enters in structure of many metalloproteins *e.g.*:
   Cerulopasmin = Plasma copper
   Erythrocuprin = Red cell copper
   Hepatocuprin = Liver copper.
   Cerebrocuprin = Brain copper.
3. Enters in structure of a chromprotein know as hemocyanin or cuprocyanin which is the equivalent of haemoglobin in red cells of invertebrate and snak.
4. Enters in and is essential. for activity of ceratin enzymes *e.g.* ascrobic acid oxidase, tyrosinase, cytochrome oxidase, uricase, iodine peroxidase and superoxide dismutase.

Requirements: 2.5 mg/day.

Sources: Nuts, liver, kidney and dried legumes.

N.B.: Milk is a poor source for iron.

Metabolism:

– Absorption of copper occurs in small intestine. The cupric ions Cu^{2+} are insoluble. Certain carrier low M.W. keep it soluble in water at intestinal pH is present in saliva and gastric juice of human. It complexes with Cu^{2+} to keep it soluble. In the intestinal mucosa copper is bound to a binding protein called "metallothionein".

– Copper in plasma binds to amino acids (especially histidine) and serum albumin. Plasma copper is rapidly removed from the circulation by the liver.

Plasma → 90% carried in cerulopasmin (α 2 glob glycoprotein)

→ 10% carried in albumin.

RBC → band to erythrocuprin (superoxide dimutase)

In the liver copper enters one of two routes:

1) Excretion in bile.
2) Integration into ceruloplasmin. Ceruloplasmin contains 6-8 atoms of copper, half as (Cu^{2+}). Ceruloplasmin copper is not available for exchange with copper ions or bound copper (it is not the transport form of copper).

Normal plasma level of copper is 100 μg/100 ml, in RBC, 100 μg/g.

a) Copper toxicity may occur due to excess intake, it is manifested by green diarrhea, green saliva and acute haemolysis.
b) Menske's disease. This is a defective absorption of copper. The defect occurs at the transport of copper from the intestinal serosa to plasma.

C- Willson's disease: (Hepato-lenticular degeneration).

* This is a disease characterized by:

1. Increased copper absorption from intestine.
2- Increased deposition of copper in liver which may result in liver cirrhosis.
3- Deposition of excessive amounts of cooper in lenticular nucleus of brain with mental disturbances.
4- Low levels of copper and of ceruloplasmin in plasma.
5- Deposition of copper in kidney leading to increased urinary excretion of amino acids ((alninoncidurin) and maybe of glucose (glucosurin).

Excretion → in stool through bile.

ZINC

Function:

1) It helps crystallization and storage of insulin in β-cells of the pancreas.
2) Component of some enzymes *e.g.* carbonic anhydrase, carboxypeptidase, retinal reductase and alcohol dehydrogenase.
3) Important for wound healing.

Sources:

Animal protein, citrus fluid and leafy vegetables.

Metabolism:

- Zinc is absorbed in the intestine by the help of a zinc binding factor, then binds inside the intestinal cell to zinc binding protein that transferes it to albumin.
- Copper interfere with zinc absorption by competing for the biniding sites on albumin. Also high plasma calcium prevents binding of zinc to albumin. Phytic acid complexes with zinc and prevents its absorption.
- In high zinc intake, it is bound by liver metallothionein.

Zinc deficiency occurs due to:

1- Malabsorption. 2- Sickle cell disease.

- In zinc deficiency there is multisystem disfunction due to its presence in many enzymes.

Excretion:

1- Stool. 2- Urine. 3- Sweat.

MANGANESE (Mn^{2+})

Function:

1. Component of many enzymes *e.g.* arginase, carboxylases and superoxide dismutase.
2. Important for growth.
3. Important for the metabolism of nervous system.
4. Important for spermatogenesis and ovulation.

Sources: Animal meats.

Metabolism:

Absorption of Mn^{2+} occurs in intestine by the help of a binding factor that makes it soluble. In the intestinal cell it is bound to a binding protein that transfers it to plasma albumin.

Excretion: mainly in stool.

CHRONIUM

Functions:

1. Important for glucose metabolism (it potentiates insulin action).
2. Important for lipoprotein metabolism.

Sources:

Yeast, grains and cereals. It is contributed in diet if cooking is done in stainless steel containers.

Metabolism:

Absorption occurs in small intestine in share with zinc. It is transported in blood to transferring.

Excretion: Mainly in urine.

SELENIUM

Functions:

- Selenium spares vitamin E or reduces its requirements. It is an essential component of glutathione peroxidase enzyme.
- This enzyme catalyzes the oxidation of reduced glutathione to oxidized glutathione as shown below:

  $2\ GSH + H_2O_2 \rightarrow G\text{-}S\text{-}S\text{-}G + 2\ H_2O$
- Reduced glutathione protects membrane lipids and other cell constituents (e.g. haemoglobin) against oxidative damage by destroying peroxide and fatty acid hyperperoxides through reactions catalyzed by glutathione peroxidase, thus:

$$\mathbf{2\ GSH + ROOH \rightarrow GSSG + ROH + H_2O}$$

FLUORINE

Function:

Involved in a modification of hydroxyapatite crystals in the enamel of the tooth to produce fluoroapatite that prevents dental caries.

Sources: Drinking water in many areas is enough.

Metabolism:

Absorption occurs in small intestine. Excess fluorine leads to fluorosis in which the teeth are brittle and mottled with white patches. Also fluorine is toxic to enzymes activated by magnesium *e.g.* enolase.

Excretion: Mainly in urine.

COBALT

Function:

It is a constituent of vitamin B_{12}. So it is essential for erythroposis. In excess administration polycythemia may occur.

MOLYBDENUM

Function:

It is a component of flavoprotein enzyme *e.g.* aldehyde oxidase, xanthine oxidase (used for uric acid synthesis) and sulfite oxidase.

DETOXICATION



DETOXICATION

— Detoxication means all chemical reactions occurring in the body to protect it against toxic substance, by this process a toxic substance is changed into non toxic, relatively harmless substance, easily excreted from the body.

— It also means a biotransformation process.

— Toxic substances may be taken (as drug, poisons), or formed in the body from non toxic materials (as those formed by bacterial action.

— The main metabolic processes involved in detoxication are:

* Oxidation
* Reduction
* Hydrolysis
* Conjugation

Most detoxication occur in the liver. The kidney may also have a role in detoxication.

A- OXIDATION

— In the most general reaction involved in detoxication process. Examples of this type of reaction:

I- Oxidation of alcohol and aldehyde to acids:

— The oxidation of alcohols to aldehyde or ketons and of aldehyde to carboxylic acid is catalysed by two groups of enzymes in the liver.

a- NAD. Linked oxidoreductase b- Aldehyde oxidase.

— The best known example of NAD linked oxidoreductases is liver alcohol dehydrogenase, which catalyzes the reaction presented in Fig.

$$\underset{\text{Ethanol}}{CH_3CH_2OH} + NAD \xrightarrow[\textit{dehydrogenase}]{\textit{alcohol}} \underset{\text{Acetaldhyde}}{CH_3\overset{O}{\overset{\|}{C}}\text{-}H} + NADH + H^+$$

- Liver aldehyde dehydrogenase catalyzes the oxidation of a number of aldehydes that is, formaldhyde and acetaldhyde, to their acids.

$$CH_3\text{-}\overset{O}{\overset{\|}{C}}\text{-}H + NAD + H_2O \xrightarrow[\text{dehydrogenase}]{\text{aldehyde}} CH_3\overset{O}{\overset{\|}{C}}\text{-}OH + NADH + H^+$$

Acetaldehyde → Acetic acid

- Aldehde oxidase is a metallofluvoprotein containing iron, molybdenum and FAD and catalyzes the oxidation of benzaldehyde to benzoic acid.

$$\text{Benzaldehyde (}C_6H_5CHO) + FAD + H_2O \xrightarrow[\text{oxidase}]{\text{aldehyde}} \text{Benzoic acid (}C_6H_5COOH) + FADH_2$$

II- Hydroxylation

A specific example is oxidation of tolnene.

$$C_6H_5CH_3 + NADPH + H^+ + O_2 \xrightarrow[\text{oxidase } P_{450} \text{ system}]{\text{mixed function}} H_3C\text{-}C_6H_4\text{-}OH + NADP^+$$

Many drugs are metabolized by this hydroxylation process.

- Aromatic acids are oxidized to benzoic acid or phenylacetic acid.
  These acids are then conjugated with glycine or glucurenic acid.

B- REDUCTIVE REACTIONS

Reduction as detoxication process is less commonly observed than oxidation. It is most commonly observed in aromatic nitro-compounds. The corresponding amino compounds are formed.

$$\text{Nitrobenzene} + 3NADPH + H^{+} \xrightarrow[\text{reductase}]{\text{Nitro -}} \text{Aniline} + 3NADP + 3H_2O$$

Aldehyde and ketons are reduced to alcohol by liver aldehyde or ketone reductase and reductase and the coenzyme NADH as in Fig.

$$R{-}\overset{O}{\overset{\|}{C}}{-}R^{-} \xrightarrow[\text{reductase}]{\text{Ketone} \quad NADPH + H^{+}} R{-}\underset{H}{\underset{|}{\overset{OH}{\overset{|}{C}}}}{-}R^{-} + . \; NADP$$

C- HYDROLYTIC REACTION

Some compounds may be hydrolysed before taking part in other reactions. For example: aspirin is hydrolysed into salicylic acid and acetic acid. Salicylic acid is exreted conjugated with glycine giving rise to salicyluric acid.

H_2O

Aspirin (acetylsaliglic acid) → CH_3-COOH + Salicylic acid

glycine → salicyluric acid

OH, COOH, O, C, N, H, C, H, COOH

D - CONJUGATION

It is the most common reaction for detoxication in the body.

It means coupling of the foreign substance or the toxic substance with a compound occurring normally in the body.

The chief substance used by the body for conjugation are glucuronic acid, sulphuric acid, acetic acid glycine, glutamine and cysteine.

1. Glucuronic acid:

- The conjugation reaction with glucuronic acid is one of the most common reactions, involving compounds which possess hydroxyl, carboxyl, amino, or sub-hydryl groups.
- Female sex hormones which are excreated in urine are usually found as glucuronides.
- The detoxication of bilirubin is through conjugation with glucuronic acid.
- UDP-glucuronate is the "active" form of the glucuronate for the conjugation reactions.

- The enzyme catalyzing the reactions called UDP-glucuronyl transferase, is found in the endoplasmic reticulum.

2. Sulfuric acid:

Sulfuric acid is used for detoxication of various compounds with phenolic hydroxyl groups. Indol (from intestinal putrefaction) is oxidized in the body to indoxyl, and this is conjugated (esterified with sulfuric acid to form indoxyl sulfuric acid. The potassium salt of this conjugate known as indican, is excreted in the urine.

(Indican is the potassium salts)

- Prior to sulfuric acid ester formation the sulfate undergoes "activation". The active form of sulfate is PAPS (3`-phosphoadenosine 5`-phosphosulfate).
- The sulfuric acid ester of phenol also found in the urine in small amount.

$$\text{Phenol (OH)} + HOSO_2OK \xrightarrow[\text{transferase}]{\text{Sulfo-}} \text{O-}SO_2OK$$

Potassium phenyl sulfate

3. Glycine:

- It conjugate with benzoic acid to form hippuric acid which is normal constituent in the urine.
- The quantitative ability of liver to convert a measured dose of benzoate to hippurate formerly was used as a test of liver function.
- The enzyme that catalyzes the conjugation of glycine is called acyl CoA, amino acid N-acyltransferase

Benzoic acid + CoASH (ATP → ADP + Pi) → Benzoyl CoA

Benzoyl CoA + glycine → hippuric acid + CoASH

Salicylic acid + CoASH (ATP → ADP + Pi) → salicyl CoA

salicyl CoA + glycine → Salicyluric acid + CoASH

4. Glutamic acid and glutamine:

Glutamic acid is conjugated with ammonia to form glutamine.

glutamic acid + NH_3 —(glutamine Synthase; ATP → ADP + Pi; H_2O released)→ glutamine

Glutamine is conjugated with phenyl acetic acid, and a number of drugs.

$$C_6H_5-CH_2-CO\sim SCoA + H_2N-CH(COOH)-CH_2-CH_2-CO-NH_2 \xrightarrow[\text{N-acetyl transferase}]{\text{Acyl CoA: amino acid}} C_6H_5-CH_2-CO-NH-CH(COOH)-CH_2-CH_2-CO-NH_2 + CoASH$$

phenyl acetyl glutamine

5. Acetic acid:

- It is used for conjugated of amino compounds *e.g.* conjugation with sulfanilamide.
- Acetyl CoA is the active form of acetic acid for conjugation reactions. The enzyme catalyzing the reactions is N-acetyl transferase.

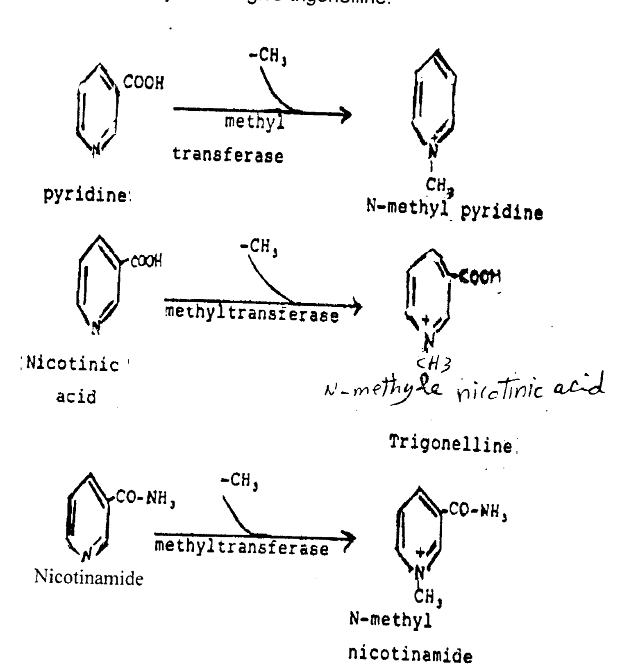
$$\underset{\text{Active acetate}}{CH_3-CO\sim SCoA} + H_2N-C_6H_4-SO_2-NH_2 \xrightarrow[\text{N- acetyl transferase}]{CoASH} \underset{\text{acetyl sulfanilamide}}{CH_3-CO-NH-C_6H_4-SO_2-NH_2}$$

- The cystine for the conjugation reactions comes from glutathione.

6. Methylgroup (methylation):

- The donor of methyl group in this process is methionine in an form "S-adenosyle methionine".

- The enzyme catalyzes this process is methyl transferase.
- Pyridine is excreted in the form of N-methyl pyridine. Nicotinic acid is methylated to give trigonelline.



7. CYSTEINE

Halogenated aromatic compounds are conjugated with cysteine, and the amino group of cysteine is conjugated with acetic acid (active acetate).

$$\text{Br-}C_6H_5 + HS-CH_2-CH(NH_2)-COOH \longrightarrow \text{Br-}C_6H_4-S-CH_2-CH(NH_2)-COOH$$

Bronobenzene + cysteine

$$\text{Br-}C_6H_4-S-CH_2-CH(NH_2)-COOH \xrightarrow[\text{N-acetyltransferase}]{CH_3-CO\sim SCoA} \text{Br-}C_6H_4-S-CH_2-CH(NH-CO-CH_3)-COOH + CoASH$$

Bronophenylmercapturic acid

HORMONES

HORMONES

Definition of hormones:

Hormones are chemical messengers or regulatory substances secreted by ductless glands 'endocrine glands'. They are transmitted via blood stream to the various tissues and organs on which they act 'target tissues'.

Like enzymes hormones also function as body catalysts, but they differ from enzymes in the following ways:

1- Not all hormones are formed of proteins.
2- They are formed in endocrine glands, far from tissues on which they act.
3- They are secreted in blood prior to use. Thus their circulating level in blood gives an indication of endocrine gland activity and target tissue exposure.

Functions of hormones:

Hormones have three major functions in the body:

1- Integrative function: They coordinate the activities of different cells in various organs of the body.
2- Regulatory function: This is achieved through their effect on different metabolic processes.
3- Morphological function: Certain types of hormones controls the rate and type of growth of the body.

Classification of hormones

Hormones can be classified according to their chemical nature, site of synthesis, location of receptors and nature of their signals and water solubility.

A- According to site of synthesis:

1- Endocrine hormones:

They are synthesized by endocrine glands and transported via blood to the target cells e.g. growth hormone, glucagone, ... etc.

2- Paracrine hormones:

They are synthesized near their targets of action. These hormones are rapidly catabolised before disseminating away e.g. plasma polypeptides as angiotensin and plasma kinins.

3- Autocrine hormones:

These hormones affects the cells which synthesis them e.g. acetylcholine, serotonine, histamine, ... Etc.

B- According to chemical nature:

1- Protein hormones:

These hormones are formed of:

a- ***Large polypeptides***: These hormones are secreted as large precursors and then processed to give the hormone e.g. insulin, parathyroid hormones, growth hormone, ... etc.

b- ***Small polypeptides***: e.g. antidiuretic hormone (ADH), adrenocorticotrphic hormone (ACTH), oxytocin, ... etc.

c- ***Glycoprotein hormones***: e.g. follicle stimulating hormone (FSH), leutenizing hormone (LH), ... etc.

2- Amino acid hormomes:

These hormones are derived from amino acids e.g.;

- Thyroid hormones and catecholamines are derived from tyrosine.
- Melatonine and serotonine are derived from tryptophan.

3- Steroid hormones:

These hormones are derived from cholesterol e.g. adrenocortical hormones, progesterone, estrogens, testosterone, ... etc.

C- According to receptor location and nature of signals:

1- Group 1 hormones:

Hormones included in this group are characterized by:

- All of them are lipophilic (hydrophopic).
- Long plasma half life which extends from hours to days.
- All of them act on intracellular receptors.
- All of them need transport protein to reach their target cells.
- Their action is mediated by forming receptor-hormone complex.

Hormones included in this group are:

- Steroid hormones: glucocortecoids, mineralocorticoids, androgens, progesterone and estrogens.
- Thyroid hormones: Thyroxine and triiodothyronine.
- Calcitriol (1, 25 dihydrocholicalciferol).

2- Group II hormones:

Hormones included in this group are characterized by:

- All of them are hydrophilic.
- Short plasma half life which extends only for minutes.
- All of them act on plasma membrane (extracellular) receptor.
- They reach their target cells without any transport protein.
- Their action is mediated by what is called second messenger as cAMP, cGMP, Ca^{++} and metabolites of phophoinositides.

Hormones included in this group are classified according their second messenger. The first messenger is the hormone itself:

- ***Second messenger is cAMP***: e.g. acetylcholine, glucagone, calcitonin, luteinizing hormone (LH), ... etc.
- ***Second messenger is cGMP***: Atrial natriuretic factor (ANF).
- ***Second messenger is calcium, phosphatidylinositides or both***: e.g. acetylcholine, α_1-adrenergic catecholamines, thyrotropin-releasing hormone, vasopressin, gastrin, ... etc.
- ***Second messenger is unknown***: Prolactin, growth hormone, insulin, ... Etc.

General mechanisms of hormonal actions

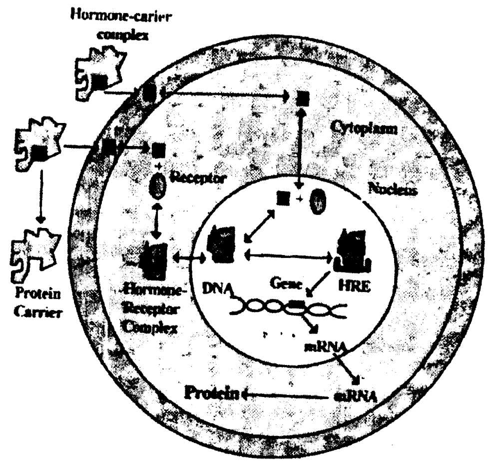
Knowing the general aspects of hormonal actions and understanding of the different mechanisms by which they act on target tissues are of vital importance in recognizing endocrine disease syndromes, that may result from hormonal imbalance and to apply effective therapy.

1- Group I hormones: (Hydrophopic hormones)

Lipophilic or hydrophopic hormones are transported by carrier proteins. They enter the cell by diffusing through the cell membrane. Inside the cell, they interact with intracellular receptors forming hormone-receptor complexs which induce different cellular responses.

Receptors for lipohilic hormones are formed from two domains. One domain is responsible for binding with the hormone and the other one binds to a specific DNA sequence.

The hormone-receptor complex undergoes temperature and salt-dependent "activation" reaction causing size, conformational and surface changes for such receptor.



Group I Hormone Mode of Action

The specific DNA sequence that bind to the hormone-receptor complex is called ***hormone response element (HRE)***. This bindng occurs only in the nucleous and cause activation or inactivation of a specific gene affecting its transcription and production of a specific protein.

Steroid and thyroid hormones are examples of this group.

II- Group II hormones: (Hydrophilic hormones)

Hydrophilic hormones freely transfere via blood stream to their target calls. They bind with the receptors on the outer surface of cell membrane (plasma membrane) initiating reactions within these cells modifying their functions.

The hormones in this group exert their actions via activation or inhibition of what is called second messenger as the hormones themselves are the first messenger.

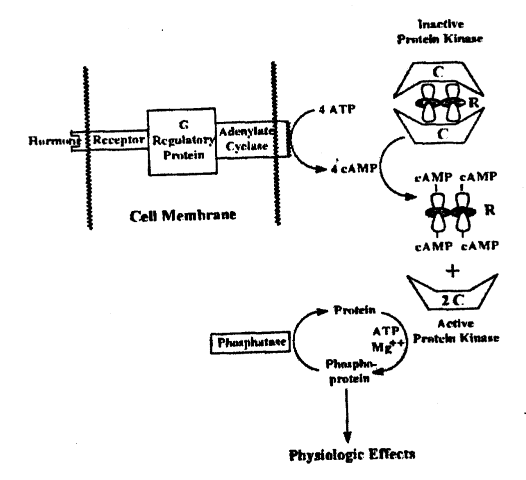
1- 3, 5 cAMP as a second messenger:

— The hormones of this group exert their action by binding with a cell membrane receptor which is large integral membrane protein. This binding is reversible. The hormone does not need to entre the cell.

— The activated receptor activates a regulatory protein (G protein) which mediates the effect of hormone to activate or inhibit adenylate cyclase enzyme.

— Activated adenylate cyclase in turn participates in formation of the second messenger i.e. 3, 5 cAMP.

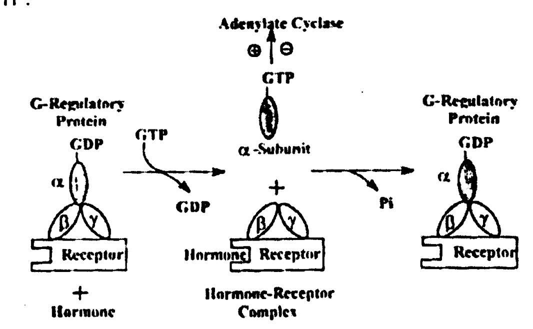
— The following items should be taken in consideration to arrive at the final effect of hormonal; action:



Group II Hormones Mode of Action
Protein kinase enzyme molecule is formed of 2 subunits i.e. R or regulatory subunit and C or catalytic subunit

A- G regulatory proteins:

- This protein is named as so because it binds guanidine nucleotides either GDP or GTP.
- It consists of three subunits which are α-, β-, and γ-subunits. Only α-subunit which binds with GDP or GTP.
- Hormone-receptor complex activates G-protein by dissociating α-subunit from β- and γ-subunits and exchanges its GDP by GTP.



Mechanism of Action of G-Regulatory Proteins

- After exerting its effect, α-subunit which contain GTPase enzyme hydrolyzes its GTP into GDP and inorganic phosphate and reassociate with β- and γ-subunits and remains inactive until reactivated again.
- There are several types of G-proteins producing different effects when activated e.g.
  - G_s stimulates adenylate cyclase.
  - G_i inhibits adenylate cyclase.
  - G_{plc} stimulates phospholipase C enzyme.

B- Adenylate cyclase enzyme:

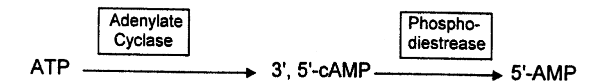
- Adenylate kinase is a large integral membrane protein which catalyzes the formation of cAMP from ATP.
- Stimulation or inhibition of adenylate cyclase depends on the type of activated G-protein by hormone-receptor complex.
- Adenylate cyclase is activated by ACTH, MSH, ADH, TSH, β-adrenergic catecholamines, glucagons and calcitonin.
- It is inhibited by actylcholine, α-adrenergic catecholamines, somatostatin and angiotensin II.

c- Protein kinase:

- cAMP-dependent protein kinase is a tetramer formed of two types of subunits:
  - Two regulatory subunits (R).
  - Two catalytic subunits (C).
- The R_2C_2 tetramer complex is inactive. On activation by cAMP the R_2C_2 tetramer complex dissociates into R_2 subunit, to which four cAMP molecules are attached, and two catalytic subunits.
- Active protein kinase transfers g-phosphate group from ATP to a specific amino acid of specific cellular protein.

D- Phosphodiestrase:

- The level of cAMP is quickly reduced as a result of its hydrolysis by cAMP-dependent phosphodiestrase enzyme.



- Phosphodiestrase is inhibited *in vitro* by high concentrations of methyl xanthines as caffeine, theophylline and theopromine found in coffee, chocolate and tea. Normal consumption of such drinks gives very low methylxanthines concentration which does not affect the enzyme.

- At therapeutic doses or concentrations resulting from normal consumption, methylxanthines exert their effects by acting as antagonists for adenosine receptors. These effects are:

System	Low concentration	High concentration
C.N.S.	Reduce fatigue	Anexiety, insomnia, tremors & convulsions
Heart rate	Increase or decrease	Tachycardia and arrhythmia
Smooth muscles	Relaxation	Relaxation

2- Cyclic GMP as a second messenger:

— Atriopeptin peptides produced in cardiac atrial tissues e.g. atrial natriuretic factor (ANF) cause natriuresis, diuresis, vasodilatation and inhibition of aldosterone secretion.

—These atriopeptins uses cGMP in a manner similar to that of CAMP. Instead of adenylate cyclase, guanylate cyclase is used.

- Nitroprusside, nitroglycerin, nitric oxide, sodium nitrite and sodium azide cause smooth muscle relaxation and are potent vasodilators.

3- Calcium or phosphoinositids as a second messenger:

— Ionized calcium is an important regulator for a variety of cellular processes including muscle contraction, blood clotting cascade, membrane excitability and enzyme activity. It also acts as intracellular messenger for many hormones.

— Some hormones like acetylcholine, ADH and α_1-catecholamines when bind to the receptors activate phospholipase C.

— Phospholipase C hydrolyses phosphatidylinositol 4, 5-biphosphate producing inositol triphosphate and diacyl glycerol. Diacyl glycerol activates protein kinas C and inositol triphosphate is an effective releaser of calcium from intracellular storage sites as sarcoplamic reticulum and mitochonderia.

Assay of hormones

1- Biologic assay:

– Hormonal activities are measured either in vitro or in vivo, using experimental animals. Although this method measures only the active hormone, yet it laks sensitivity and accuracy.

2- Chemical methods:

– These include various methods of isolation and purification e.g. gas chromatography. Column chromatography and electrophoresis. They also provide a measure of total hormone quantity but they are not application with protein hormones.

3- Radioimmunoassay:

– These techniques depend upon the competition between radio-labeled hormone (tracer), and serum hormone to be measured for specific binding protein. This binding protein is usually antibody to the hormone unlabeled (serum) hormone, competitively displaces the labeled hormone (tracer) resulting in an increase in the total activity of the unbound fraction.

– Many methods are available and different techniques are distributed commercially.

4- Enzyme-immunoassay:

– In enzyme-immunoassay technique, an enzyme is attached to hormone antibody. When the reaction occurs this enzyme is released and can be chemically determined. The concentration of the released enzyme will be proportional to that of the hormone.

Hypothalamic hormones

Hypothalamic hormones include the following:

1- Growth hormone-releasing hormone.
2- Growth hormone-release-inhibiting hormone (somatostation).
   - Both regulate secretion of growth hormone.
3- Corticotropin-releasing hormone regulates ACTH secretion.
4- Thyrotropin-releasing hormone regulates the secretion.

5- Gonadotropin-releasing hormone regulates FSH and LH secretion.
6- Prolactin releasing hormone.
7- Prolactin release-inhibiting hormone.
   - Both regulate the secretion of prolactin.
8- Melanocyte-stimulating hormone-releasing hormone.
9- Melanocyte-stimulating hormone release-inhibiting hormone.
   - Both regulate melanocyte-stimulating hormone secretion.

Regulation of secretin "Hypothalamic control":

Hormonal secretion by the pituitary gland is regulated by factors or hormones from the hypothalamus. Hypothalamus is connected directly to the pituitary by pituitary stalk.

With this stalk, a portal system of blood vessels transport hypothalamic secretion to the pituitary gland.

Pituitary hormones

The human pituitary is a reddish-gray oval structure, about 10 mm in diameter, located in the sella turcica, as an extension from the floor of the hypothalamus.

It is composed of two types of tissues of two different embryologic sources i.e. glandular or buccal and neural components.

a- Adenohypophysis (glandular or buccal component) include anterior lobe and middle or intermediate lobe.

b- Neurohypoopysis (neural component) include the posterior lobe and infundibular or neural stalk, that attaches the pituitary to the brain floor at the hypothalamus.

Anterior lobe of the pituitary

The anterior lobe, is the largest and most essential part of the pituitary. It contains three types of cells which are differentiated by their staining properties i.e. eosinophilic or acidophilic,

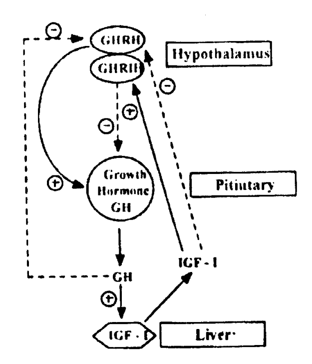
basophilic and chromophobe cells. The following hormones are secreted by anterior pituitary:

1. Growth hormone (GH): (Somatotropin)

- GH is a single polypeptide composed of 191 amino acid with a molecular weight of 22000.
- It is secreted by somatotropes (subclass of pituitary acidophilic cells). Its concentration is 5-15 mg/g of pituitary tissues.

Regulation of secretion:

- Secretion of GH is episodic and pulsatile. Its level can rise as much as 10-folds within a few minutes. It has diurnal variations.
- Secretion of GH is increased by many stimuli:
  - Sleep is one of the major causes of GH increase.
  - Stress e.g. pain, cold, apprehension and surgery.
  - Exercise severe hypoglycaemia, fasting, protein meals and amino acid argentine.
- Secretion of GH is increased by many stimuli:



Regulation of growth hormone secretion

Functions:

The growth related functions of growth hormone are primarily mediated by what is called ***insulinlike growth factor I & II***.

1- Protein metabolism:

- it is an anabolic hoprmone which helps transport of amino acids across cell membranes.
- It stimulates protein synthesis and increases body weight i.e. it causes positive nitrogen balance.

2- Carbohydrates metabolism:

- GH has an insulin antagonistic action as it increases blood glucose level by inhibiting its utilization by peripheral tissues and by stimulation of gluconeogenesis (synthesis of glucose from non-carbohydrate sources).

3- Lipids metabolism:

- GH stimulates lipolysis i.e. ubcreases liberation of free fatty acids and glycerol from triglycerides of adipose tissue.
- GH stimulates ketogenesis i.e. increases ketone bodies formation in absence of insulin as in diabetic patients.

4- Mineral metabolism:

- GH promotes positive calcium, phosphorous and magnesium balance.
- GH casuses retention of sodium, potassium and chlorides.

5- Prolactin-like effects:

- GH stimulats mammary glands and lactogenesis.

Somatomedins:

- Growth hormone stimulates the production of somatomedins from the liver and kidney.
- Somatomedins are responsible for the anabolic action of growth hormone.
- Five types of somatomedins are known.

Somatostatin: "Growth hormone release inhibiting hormone"

- Isolated from hypothalamus.
- It is also secreted from other tissues as δ-cells of Langerhans.
- It is a tetradecaptide with a disulphide bridge.
- It inhibits growth hormone release by inhibiting Ca^{++} mobilization.
- It inhibits release of insulin, glucagons, TSH, FSH and ACTH.

Tumours of growth hormone secreting cells increase GH leading to:

- If it occurs before epiphyseal closure it causes **Gigantism** which is characterized by generalized over growth of long bones, so that a
  1- The patients span is more than his hight.
  2- The lower segment is longer than upper segment of the body.
- If it occurs after epiphyseal closure it causes ***Acromegally*** which is characterized by:
  1- Big Skul with protruded mandible (prognathisim) and prominent mastoid process and supra-orbital ridges.
  2- Enlarged tongue, lips, nose, ears and viscera (visceromegally).
  3- Enlarged hands (spade hands) and feet.

Deficiency of growth hormone: Pahypopituitrism leads to ***dwarfism*** characterized by short stature and childish look with normal mentality and sexuality.

2- Pituitary tropins:

Anterior pituitary produces what is called ***tropic hormones*** which influence the activity of other endocrine glands. They are carried by blood to other endocrine (target) glands, to aid in maintaining their functions.

A- Prolactin: (Lactogenic H., Luteotropic H or Mammotropin)

- Prolactin is a protein hormone formed of 198 amino acids with a molecular weight of 23000.
- Prolactin is produced by lactotropes (acidophilic cells).

Functions:

1- It is responsible for initiation and maintenance of lactation.

2- It activates the corpus luteum and stimulates continuous progesterone production, hence the name Luteotropic hormone.

3- It has anabolic effects-like these of growth hormone-but less active. It is increased during pregnancy, and together with other anabolic effects, it helps development and growth of mammary glands.

Tumours of prolactin secreting cells increases prolactin leading to:

- ***Amenorrhoe*** (cessation of menstrual cycle) and ***galactorrhea*** (breast secretion) in women.
- ***Gynaecomastia*** (large breast) and ***impotence*** in men.

Decreased prolactin secretion leads to failiure of lactation.

B- Gonadotropins:

There are tropic hormones that influence the function and maturation of testis and ovary.

They are glycoproteins in nature, and their molecular weight ranges from 25000 and 40000. They are formed of two chains;

- α- Chain which is identical in all the three types.
- B- chain which differe in each one as it determines the specific biologic activity.

Gonadotropins include follicle-stimulating hormone (FSH), luteinizing hormone (LH) and human chorionic gonadotropi (hCG).

1- Follicle-stimulating hormone (FSH):

Functions:

1- In females, it promotes follicular growth and prepares the follicles to the actions of LH.
2- In males, it stimulates tesicular growth.
3- It, also b stimulates synthesis of androgen-binding protein that binds and transports testosterone to the seminefrous tubules and epididymis where it stimulates spermatogenesis.

2- Luteinizing hormone (LH):

Functions:

1- In females it stimulates final maturation of graffian follicles, ovulation and development of corpus luteum. Osetrogen and progesterone secretions are also stimulated.
2- In males, it stimulates production of testerone by Leydig cells of the testis and support spermatogenesis.
3- In males, it is responsible for development of secondary se characters and accessory se organs as prostate and seminal vesicles.

3- Human chorionic gonadotropin (hCG):

Function:

This hormone is closely related to LH. It increases in blood and urine shortly after implantation. Its detection is the basis of pregnancy tests.

C- Thyrotropic hormone: Thyroid-stimulating hormone (TSH)

TSH is a glycoprotein formed of α- and β-chains. Its molecular weight is 30000.

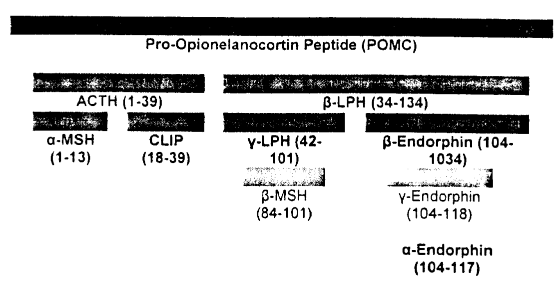
Functions:

TSH increases thyroid growth, and production of thyroid hormones. Injection of thyrotropin will lead to manifestations of

hyperthyroidism. Decreased TSH secretion leads to manifestations of ***pituitary myxaedema***.

D- Pro-opiomelanocortin peptide family: (POMC)

POMC protein is synthesized in the anterior pituitary as a precursor molecule of about 285 amino acids. Its cleavage produces ACTH and β-lipotropin. The cleavage of ACTH gives α-MSH and CLIP, while β-lipotropin produces γ-LPH and β-endorphin. Breakdown of γ-LPH gives β-MSH and β-endorphin gives γ-endorphin which ends with α-endorphin.



Pro-Opiomelanocortin (POMC) Cleavage Products

1- Adrenocorticotropic hormone: (ACTH)

ACTH is a polypeptide composed of 39 amino acids. Its molecular weight is 4500.

Functions:

1- It helps the synthesis and release of corticosteroid hormones by the supra-renal cortex.
2- It has also tropic effects on the adrenal tissues i.e. increases growth and weight of adrenals.
3- It has a weak MSH-like action.

Excess production of ACTH produces ***Cushing's Syndrome.***

The excess of ACTH causes increased production of glucocorticoids, mineralocorticoids, and anderogens. This leads to:

1- Retention of Na^+, Cl^- and water leading to hypertension.
2- Increased excretion of potassium, phosphorus and nitrogen.
3- Elevation of plasma glucose level (***diabetes Mellitus***).
4- Evevated plasma fatty acids with a peculiar distribution of fat (truncal obesity) and muscle atrophy.
5- Hyperpigmentation due to its MSH-like action.
6- Polymorphnuclear leucoctosis, eosinopenia and lymphopenia.

2- β-lipotropin hormone: (β-LPH)

B-Liptropin hormone is a polypeptide formed of 91 amino acids. It is only found in the pituitary gland then it is rapidly converted to γ-lipotropin (γ-LPH) and β-endorphin.

Functions:

- It causes lipolysis and fatty acid mobilization.
- It mainly serves as a precursor for β-endorphin.

3- Melanocyte stimulating hormone: (MSH)

See middle lobe pituitary gland.

4- Corticotropinlike intermediate lobe peptide: (CLIP)

Not detected in human but found in animals with well developed middle lobe.

5- Endorphins:

β-endorphin (31 amino acids) is the parent precursor for α-endorphin (14 amino acid) and γ-endorphin (15 amino acids). These endorphins bind to the central nervous system receptors and play a role in control of endogenous pain. Their analgesic power is 18-30 times higher than morphin.

Middle lobe of the pituitary

— The middle lobe of the pituitary secretes melanocyte stimulating hormone (MSH).

— Three different MSH peptides, α, β, and γ are derived from POMC peptide. The α-MSH contained 13 amino acids in exactly similar sequence as the first 13 amino acids of ACTH. The β-MSH contained 18 amino acids. In humans, the circulating MSH activity is contained within the larger peptide γ- or β-LPH.

— Both α, and β-MSH are structurally similar to ACTH, and hence ACTH has small but definite MSH activities.

Functions:

— MSH stimulates melanogenesis by causing dispersion of intracellular melanin granules leading to darkening of the skin human skin.

— Patients with insufficient production of glucocorticoids (***Addison's disease***), have hyperpigmentation. This is not only due to increased ACTH but also due to increased β- and α-LPH with their associated MSH activity. In patients with panhypopituitarism pigmentation does not occur.

Posterior lobe of the pituitary

— Hormones of the posterior lobe of the pituitary are primarily produced by the neurosecretory neurons.

— Vasopressin (ADH) is secreted from the supraoptic nucleous in the hypothalamus and stored in the posterior piturity in association with a specific protein called Neurophysin I (mol. w. 19000).

— Oxytocin is secreted from the paraventricular nucleous and stored in the posterior pituitary in association with a specific protein called Neurophysin II (mol. w. 21000).

Both of these hormones are nonapeptides formed of 9 amino acids and contain cysteine at position 1 and 6 linked by an S-S bridge.

1- Vasopressin (antidiuretic hormone ADH):

- It exerts an antidiuretic effect. It increases the permeability of cells of distal convoluted tubules to water. Normally these tubules pass via hypertonic interstitial tissue and their cells are impermeable to water. Thus ADH hormone helps the diffusion of water from the filtrate passing in the tubules to the surrounding interstitium.

- Urine volume is decreased from 30 liter/day in absence of ADH to 05-1 liter in its presence.

- Lack of ADH leads to ***Diabetes Insipidus*** which is characterized by excessive urine excretion. The specific gravity of this urine is very low nearly similar to water i.e. about 1000.

2- Oxytocin:

This hormones is similar in structure to ADH, and each of them may exhibit some of the effects of the other.

It stimulates contraction of myoepithelial cells surroundinf mammary alveoli squeezing milk into the alveolar ducts leading to milk ejection.

It induces strong uterine contractions, and in pharmacologic doses, it is used to induce labour in humans.

Thyroid gland

- Thyroid gland is composed of two lobes, one on each side of the trachea, connected by the middle isthmus, making the whole gland more or less H-shaped in appearance.

- Histologically, it is formed of closely packed thyroid follicles, filled with proteinaceous material, the colloid.

- The thyroid hormones are synthesized and secreted by the thyroid gland.

Biosynthesis of thyroid hormones:

Thyroid gland has a marked capacity to concentrate iodine absorbed against electrochemical gradient. This is an energy-dependent process, that requires ATP and active sodium pump.

1- Within the thyroid, iodide is oxidized to a higher valency state, by a heme-containing peroxidase enzyme. This is an essential step before iodide could be incorporated into thyroid hormones.

2- Thyroglobulin is a glycol protein (mol.w. 660000), synthesized in the basal part of the cells of thyroid follicles and is stored in the extracellular colloid. It contains 115 tyrosine residues per molecule. These tyrosine molecules are iodinated to yield thyroid hormones.

3- Oxidized iodide reacts with tyrosine molecules in the thyroglobulin, at first in the 3^{rd} position producing monoiodotyrosine (MIT) and then in the 5^{th} position giving diiodotyrosine (DIT).

4- Oxidative coupling within the thyroglobulin of two DIT molecules will from T_4 (throxine), while coupling of one DIT and one MIT produces T_3 (Triiodothyronine) or 3,5,3' triiodothyronine.

5- The formed thyroid hormones (T_3 and T_4) remain as apart of thyroglobulin, till TSH stimulates hydrolysis of this protein. This hydrolysis is achieved by acid proteases and peptidases, resulting in discharge of T_3 and T_4 into the blood.

6- Hydrolysis of thyroglobulin is also associated with release of the inactive MIT and DIT into the circulation. The deiodinase enzyme system is present in liver and kidney as well as in the thyroid, removes iodine from these inactive compounds thus preserving iodide for further thyroid hormones synthesis.

Tyrosine → Monoiodotyrosine (MIT) → Diiodotyrosine (DIT)

Oxidative Coupling

MIT + DIT → Alanine + Triiodothyronine (T_3)

DIT + DIT → Alanine + Tetraiodothyronine (T_4) Thyroxine

Blood transport:

In the blood T_4 and T_3 circulate bound to two specific proteins.

1- Thyroxine binding globulin (TBG) which is synthesized in the liver and decreases in advanced liver and renal diseases. Its affinity to T_4 and T_3 is 100 times more than TBPA.

2- Thyroxine binding prealbumin (TBPA), also synthesized in liver.

3- About 0.05% of circulating thyroid hormones, is "free" or in the "unbound state" and these are the metabolically active hormones in the plasma.

Function of thyroid hormones:

T_4 and T_3 are the major thyroid hormones. Although T_3 level is much lower than that of T_4, yet T_3 is loosely bound to plasma proteins and is 2.5 times more active than T_4. T_3 has also amore rapid onset of action.

1- Thyroid hormones increase oxygen consumption and activate the process of oxidative phosphorylation all over the body.
2- They increase the basal metabolic rate.
3- In physiologic states they enhance general protein synthesis and cause positive nitrogen balance.
4- In large doses it exerts a catabolic effect inhibiting protein synthesis.

Control of secretion:

Thyroid gland activity is regulated by TSH. There is feedback regulatory mechanism between TSH and thyroid hormones.

Abnormalities of thyroid function:

A- Hypothyroidism:

It is characterized by:

1- Cretinism, results from incomplete development or congenital absence of thyroid gland. It is characterized by failiure of normal physical, sexual and mental growth.
2- Childhood hypothyroidism "Juvenile myxoedema". Appears later in life and is less severe. There is short stature but no mental retardation.
3- Myxoedema or hypothyroidism in adults. Characterized by non-pitting oedema with low basal metabolic rate and body temperature.
4- Simple (endemic) goiter. It is caused by inadequate iodine supply in the diet.

B- Hyperthyroid states: (Toxic Goiter, Grave's Disease)

— It differs from simple goiter in that the enlargement of the gland is associated with increased production of thyroid hormones. There is nervousness, fatigability, loss of weight, increased body temperature, increased heart rate, increased basal metabolic rate ... etc.

Calcitonin "Thyrocalcitonin":

— Another hormone, which is involved in the process of calcium regulation, secreted by parafollicular C-cells of the thyroid gland. It is a peptide, composed of 32 amino acids and having a molecular weight of 23600.

Functions:

— It is a calcium lowering hormone, having metabolic effects opposite to these of parathyroid hormone, though not acting in the same way.

— Calcitonin secretion is stimulated by high plasma ionized calcium. It increases deposition of calcium in bones.

Parathyroid glands

— Parathyroids are four small glands, closely associated with the thyroid, weighning around 0.3 gm.

— They secrete parathyroid hormone or parathormone (PTH).

Chemistry:

- Parathyroid hormone is a single-chain polypeptide, containing 84 amino acids with a molecular weight of 9500.
- It is initially synthesized as a preprophormone, containing 115 amino acids. This precursor loses 25 amino acid to yield the prohormone containing 90 amino acids. In Golgi apparatus another 6 amino acids are removed to give the mature PTH.

Functions:

The primary functions of parathyroid hormone are concerned with regulation of calcium and phosphorus metabolism.

1- Actions on GIT:

It increases the absorption of calcium from the intestine this appears to be secondary to its effect of increased production of 1,25 $(OH)_2D_3$.

2- Actions on the kidney:

PTH increases renal tubular reabsorption of calcium and decreases reabsorption of phosphorus leading to hypocalciuria and hyperphosphaturia.

3- Actions or bones:

PTH increases bone resorption, leading to release of calcium and phosphorus from bones. Also collagenase, lysosomal enzymes and hydroxyproline are released.

PTH increases the concentration of both lactic and citric acids in bones thus helping their dissolution. However, the amount of acids produced are insufficient to account for such degree bone resorption.

In general, the PTH administration leads to:

1- Rise in serum calcium.
2- Decrease in serum phosphorous.
3- Rise in alkaline phosphatase activity.
4- Activation of 25 $(OH)D_3$ into 1, $25(OH)_2D_3$, in the kidney.

Control of activity:

The hormone is not stored in parathyroid glands. It is synthesized and secreted continuously.

The secretion of parathyroid hormone is regulated by a negative feedback mechanism in relation to the levels of ionized plasma calcium. PTH secretion is decreased abruptly by injection of calcium ions.

Abnormalities in parathyroid functions:

I- Hypoparathyroidism:

Usually, this results from accidental removal during thyroidectomy. PTH deficiency causes occurrence of tetany (muscle cramps) which might lead to death in severe cases. The condition is characterized by:

1- Low serum calcium and high serum phosphorous.
2- Low urinary calcium and phosphorus.

II- Hyperparathyroidism:

Increased production of parathyroid hormone e.g. by a tumor may result in:

1- Decalcification of bones, causing deformities and spontaneous fractures.
2- Deposition of calcium in soft tissue. Renal stones might be formed.
3- High serum calcium and low serum phosphorus.
4- Increased urinary calcium and phosphorus.

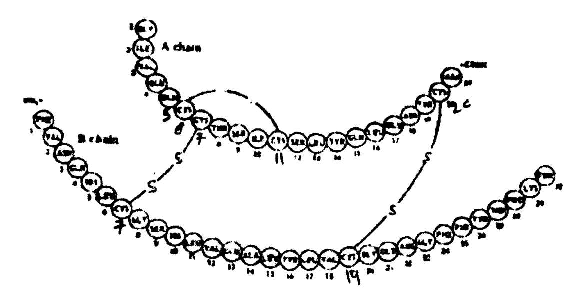
The pancreas

The endocrine functions of the pancreas are located in the islets of langerhans. Two hormones are produced by these islets i.e. insulin and glucagons.

Insulin

- Insulin is a protein hormone, that has been isolated from the pancreas and prepared in crystalline form. Crystallization of insulin requires zinc and this appears to be constituent of stored pancreatic insulin.
- Insulin molecule (molecular weight 36000) is composed of 2 polypeptide chains (51 amino acids):

1- Chain A composed of 21 amino acid.
2- Chain B composed of 30 amino acids.



The two chains are connected by two disulfide bridges:

1- One between amino acid number 7 of chain A and number 7 of chain B.
2- The second between amino acid number 20 of chain A and number 19 of chain B.

There is also a disulfide bond between amino acids number 6 and 11 of chain A.

In the B-cells. Insulin is synthesized as a precursor i.e. Proinsulin.

Biosynthesis:

→ Insulin is synthesized in the polysome (protein synthesis) as a preprophormone containing 109 amino acids with a mol. w. of 11500.

→ In the cisternae of the rough endoplamic reticulum, it loses 23 amino acids giving proinsulin.

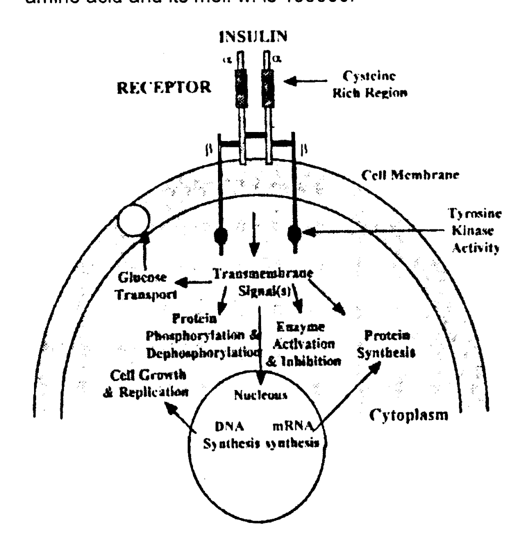
→ Proinsulin contains 86 amino acids its mol. w. is 90000. It is formed of β chain - connecting peptide (C - peptide) - α Chain.

→ By the action of proteolytic lysosomal enzymes, proinsulin is converted into insulin and physiologically inactive C - peptide.

This free, inactive C-peptide is ultimately secreted in equal molar ratio with insulin.

Mechanism of action of insulin:

- Insulin receptor is a specific glycoprotein with which insulin bind to exert its action.
- The receptor is formed of a heterodimer. Each unit of the dimer is formed of two subunits i.e. α- and β-subunits. The two units of dimer as well as the two subunits are linked together by disulphide bridge giving what can called a heterotetramour.
- Human Insulin receptor precursor is formed from 1382 amino acid and its mol. w. is 190000.



Relationship between insulin receptor and insulin action

In the rough endoplasmic reticulum this precursor undergoes cleavage to give the mature human insulin receptor as a single-chain peptide which undergoes extensive rapid glycosylation in the golgi region. Removal of sialic acid and galactose decreases both insulin binding and insulin action.

- Insulin receptors have a half life of 7-12 hours, therefore these receptors are in acontinuous state of synthesis and degradation.
- The human insulin receptor gene is located on chromosome 19. These receptors are found in most cells in a concentration of up to 20000 per cell. They are formed of two subunits.
    - ***a- Subunit***: its mol.w. is 135000 and is entirely extracellular. It binds insulin, propably, via the cysteine rich domain.
    - ***b- Subunit***: Its mol.w. is 95000 and is a transmembrane protein that performs the second major function of insulin i.e. signal transduction. The cytoplasmic portion of this subunit has tyrosine kinase activity and an autophosphorylation site. Thes are responsible for signal transduction and insulin action.

Events of insulin biding: The following events occurs when insulin binds with the receptor:

1- The receptors undergoes conformational changes.
2- The receptors cross-link together forming aggregates.
3- The receptors become enternalised : Enternalisation of the receptor represents a means of controlling the receptor concentration and turnover.
4- One or more signals are generated.

* Increased plasma insulin levels as in obesity and acromegally cause decreased number of insulin receptors and the target tissues become less sensitive to insulin.

Functions of insulin:

1- On carbohydrates:

Insulin is "Hypoglycaemic agent" i.e. lowers blood glucose. This is achieved through the following actions.

a- It helps transport of glucose across the cell membrane except liver and brain cells which are freely permeable to blood glucose.

b- It helps oxidation of glucose in glycolysis (anaerobic pathway for glucose oxidation) and Kreb's cycle (aerobic pathway for glucose oxidation) by increasing activit and amount of glucokinase, phosphor-fructo kinase and pyruvate kinase.

c- It helps formation of glycogen from glucose (glycogenesis). This action is mediated by indirect activation of glycogen synthase via activation of phosphodiestrase which converts 3' 5' cAMP into AMP.

d- It helps lipogenesis i.e. formation of fats from glucose by:

- Providing active acetate and reduced NADP needed for fatty acid synthesis. It also provide glycerol required for triglycerides.
- It stimulate acetyl CoA carboxylase which is key enzyme for lipogenesis.

2- On lipids:

a- Insulin stimulates lipogenesis and inhibits lipolysis (breakdown of fats), thus level of fatty acids in blood is lowered.

b- It inhibits the formations of ketone bodies, and cholesterol.

3- On proteins:

Insulin is an anabolic hormone:

1- It stimulates protein synthesis from amino acids.

2- It produces positive nitrogen balance.

3- It inhibits protein catabolism by inhibiting the process of oxidative deamination thus decreasing formation of glucose from amino acids.

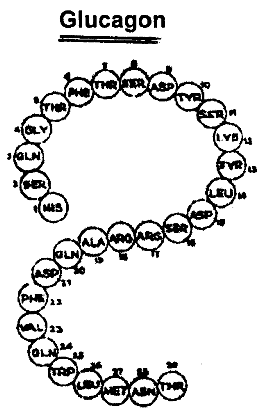
Insulin deficiency:

Insulin definiency or resistance to action of insulin causes ***diabetes mellitus***.

Catabolism of insulin:

Primarily in the liver and kidney, the enzyme glutathione insulin transhydrogenase, transfer hydrogen from reduced glutathione to S-S bonds of insulin, thus chain A and B are separated. These chains are further hydrolyzed by proteolytic enzymes.

Glucagon



- Produced by α-cells of islets of Langrehans.
- It is single polypeptide chain, composed of 29 amino acids, having a molecular weight of 3500. It is secreted as a preprohormone which undergoes a series of selective proteolytic cleavages to give the mature hormone.

- It contains no –S-S- bridges and needs no zinc for its crystallization.
- Its secretion is stimulated by low plasma glucose and inhibited by high plasma insulin level.
- On the contrary, it is a "Hyperglycaemic agent" i.e. raises blood glucose.

Functions:

1- Glucagon stimulates glycogenolysis i.e. breakdown of glycogen into glucose. This action is mediated by 3'5' cAMP.

2- It stimulates lipolysis, thus increasing the level of free fatty acids in the blood.

The adrenals

Adrenals involve, the adrenal cortex and adrenal medulla.

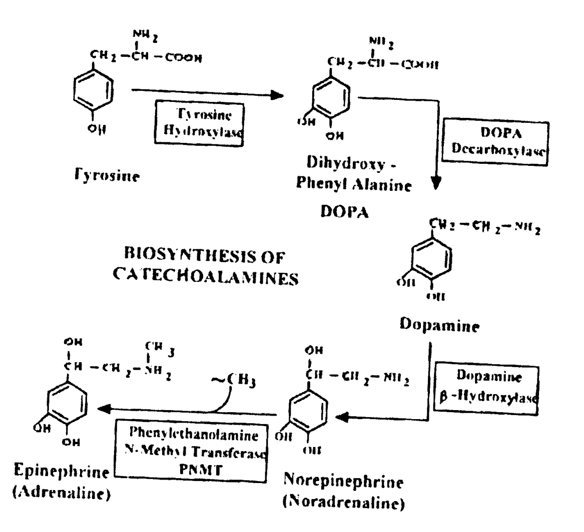
The adrenal medulla

The adrenal medulla is a derivative of the sympathetic portion of the autonomic nervous system. ***Catecholamines*** hormones secreted by adrenal medulla are ***epinephrine (adrenaline)*** and ***norepinephrine (noradrenaline)***. The adrenal medulla contains ***cromaffin granules*** which are capable of biosynthesis, storage, uptake and secretion of catecholamines.

Catecholamines
(Epinephrine and Norepinephrine)

Biosynthesis:

Catecholamines (epinephrine and norepinephrine) are synthesized from the amino acid tyrosine.



Mecanism of action:

These hormones act through two major types of receptors:

α- ***Adrenergic receptor***: There are two subtypes; α_1- and α_2-adrenergic receptors. Catecholamines through these receptors stimulate cyclic AMP dependent protein kinase and inhibit adenyl cyclase.

β- ***Adrenergic recptors***: There are also two types, β_1- and β_2-adrenergic receptors. Catecholamines through them increase cyclic AMP.

Functions:

Epinephrine – in general – duplicates the effects of sympathetic nerous system. It is necessary to provide a rapid physiologic response to emergencies as cold, shock, fatigue ... etc. In this sense, it is termed the hormone of "fight" or flight".

1- On the cardiovascular system:

Both epinephrine and norepinephrine lead to an elevation in blood pressure, more marked in cases of norepinephrine as a result of their actions on heart and blood vessels.

2- On the smooth muscles:

Epinephrine causes relaxation of muscles of stomach intestine, bronchioles and urinary bladder, together with contraction of sphincters.

3- Metabolic effects:

a- carbohydrates:

Epinephrine elevates blood glucose level, since it stimulates glycogenolysis, in both liver and muscles. This action is mediated by 3' 5' cAMP.

b- Lipids:

Epinephrine enhances lipolysis, thus increasing level of free fatty acids in blood. This is due to stimulation of lipase enzyme, an action which is mediated also by 3' 5' cAMP.

c- Proteins:

Catecholamines have a little catabolic effect on proteins as they enhances gluconeogensis.

d- Epinephrine has a direct inhibitory effect upon insulin release by the pancreas. It thus serves as an emergency hormone by:

i- Rapiidly providing fatty acids which are the fuel for muscle action.

ii- Rapidly mobilizing glucose via glycogenolysis.

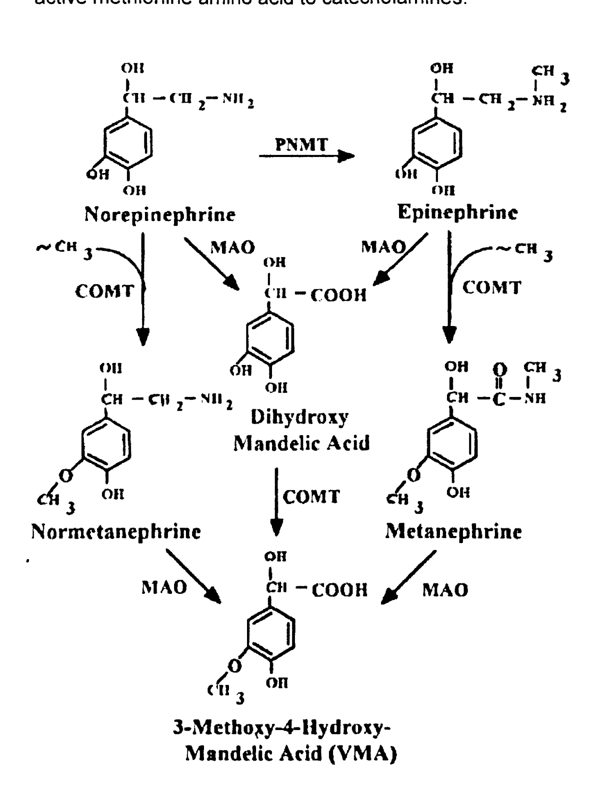
iii- Decreasing insulin secretion, thus preventing glucose from being taken by peripheral tissue and keeping it for CNS actions.

Catabolism of catecholamines:

Catecholamines are catabolized by two enzymes:

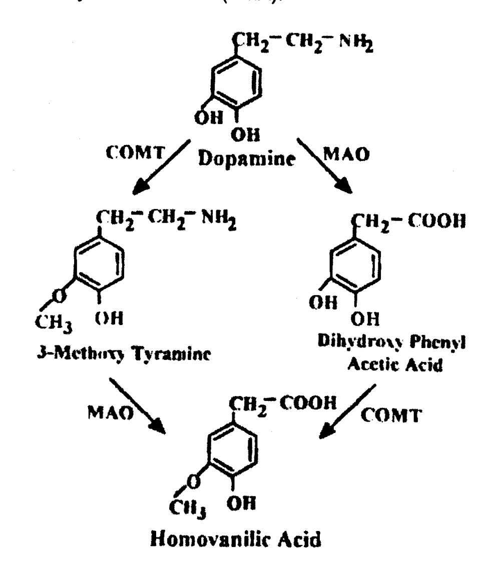
1- Monoamine oxidase (MAO) catalyses oxidation of side chains.

2- Catechol-O-methyl transferase (COMT) catalyses the transmethylation reactions. i.e. transfer of CH_3 groups from active methionine amino acid to catecholamines.



The actions of both enzymes leads to formation of group of compounds which are extracted in urine e.g.

1- 3, 4-dihydroxy mandelic acid.
2- Metanephrine.
3- Normetanephrine.
4- Vanily mandelic acid (VMA).



- Urinary excretion of VMA is considered as a monitor of adrenal medullary function.

Pheochromocytoma:

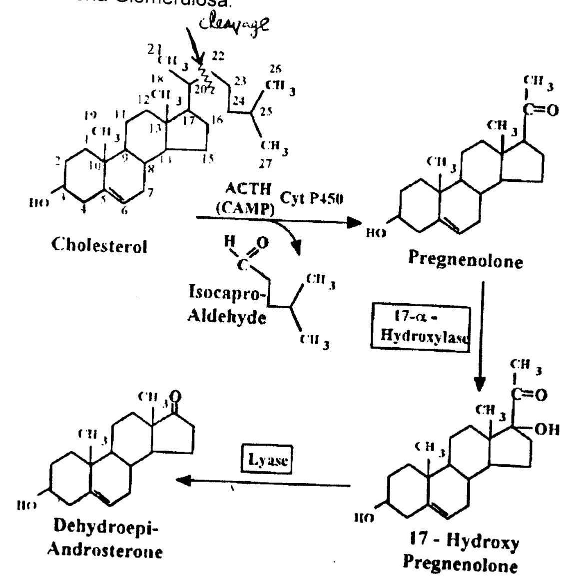
Tumors of the suppra-renal medulla characterized by symptoms of hyperactivity. Like intermittent hypertension, and increased excretion of VMA in urine.

The adrenal cortex

- The outer portion of the adrenal gland, the adrenal cortex is essential for life. Its embryologic origin is the mesoderm while ectoderm is the embryologic origin of adrenal medulla.

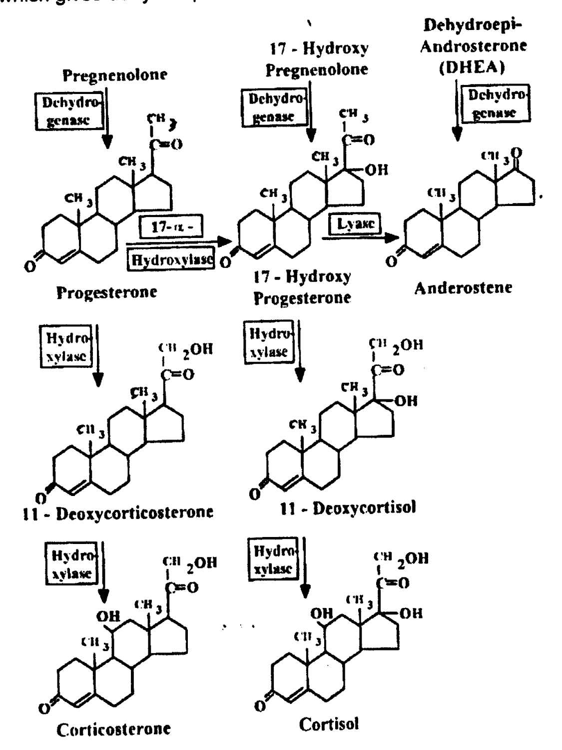
- All hormones produced by the adrenal cortex are steroid derivatives, having the perhydro-cycleptano-phenanthren nucleus. These hormones fall into three general classes.

1- Glucocorticoids secreted from Zona Fasciulata.
2- Mineralocorticoids secreted from Zona Glomerulosa.
3- Sex hormones, androgens and estrogens secreted from Zona Glomerulosa.



Biosynthesi of adrenal hormones:

- Active acetate is the precursor of all steroid hormones. This gives cholesterol which is converted to pregnenolone.
- Pregnenolone is converted either to 17-hydroxy pregnenolone which gives dehydroepiandrosterone or progesterone.



- Pregnenolone and 17-hydroxypregenolone are converted to a variety of active hormones by specific oxygenases and dehydrogenases which require molecular oxygen and NADPH.
- The result of these enzymatic reactions is addition of hydroxyl or ketonic groups at C_{11}, c17, or C_{21} position.
- In general C_{21} hydroxylation is necessary for both glucocorticoid and mineralocorticoid activities.

Catabolism and excretion of steroid hormes:

Steroid hormones are catabolised in the liver by reduction of the double bond in the steroid nucleous and the ketonic groups at C_3.

The produced metabolites become conjugated with glucuronic acid or sulphated (minimal pathway).

Most of these metabolites are excreted in bile, however, only 20% are excreted in stools. The rest becomes reabsorbed and 70% of them are excreted in urine and 10% in sweat.

Metabolic functions:

A- The glucocorticoids:

The steroids containing OH or C = O at the C_{11} position and OH at C_{17} have glucocorticoid activities e.g. corticosterone, 11-dehydrocorticosterone, cortisone and hydrocortisone (cortisol). The most important glucocorticoids are cortisol and corticosterone.

They act as other steroids by modification of RNA and enzyme synthesis in target tissue. They control carbohydrates, lipids and protein metabolism:

1- <u>Carbohydrates</u>:

- They decrease glucose uptake, by inhibition of glucokinase enzyme.
- They inhibit glycolysis.

- They help gluconeogenesis, since the enzymes which are involved in amino acid conversion into glucose are increased.
- The glycogen deposition is increased.
- In general, the actions of gluocortiicoids, are metabolically antagonistic to insulin, with consequent rise in blood glucose level "hyperglycaemia"

2- Fats:
- Glucocoriticoids enhance lipolysisi (breakdown of triglycerides of adipose tissue) particularly in the extremeties. The level of free fatty acids is increased in blood.
- They help gluconeogenesis from liberated glycerol of hydrolysis of triglycerides.

3- Protein:
- In physiological concentrations glucocorticoids have anabolic action. Protein synthesis is depressed, whereas protein catabolism is increased.
- This leads to increased extraction of NPN compounds in urine and a state of negative nitrogen balance.

4- Other effects of glucocorticoids include:

a- Anti-inflammatory effects:

They are used in treatment of collagen diseases as rheumatoid arthritis.

b- Immunosuppressive effects:

Cortisol decreases the immune respone associated with infection, and allergic states.

c- Stress

Glucocorticoids are elevated several folds in response to acute stress to reverse the decreased blood pressure resulting from emotional or surgical shock.

d- Miscellaneous effects:

- They increase lung surfactant, consequently they are used in treatment of ***respiratory distress syndrome (RDS)*** in newly born premature infants.
- They stimulate the secretory functions of gastrointestinal tract (GIT) increasing secretion of HCl, pepsinogen, trypsinogen ... etc. Adminstration of glucocorticoids enhances GIT ulceration e.g. peptic ulcer.

B- Mineralococrticoids:

The most potent mineralocorticoid is aldosterone, its pathway of synthesis need C_{18}-hydroxylation.

With exception of androgens, all corticosteroids increase the absoption of Na^+ and Cl^- by the renal tubles i.e. reduce their excretion. However, glucocorticoids e.g. cortisol have the least sodium-retaining action while aldosterone is at least 1000 times as effective as cortisol in this respect. Mineralocorticoids have the following actions:

1- Sodium-retaining actions:
   Reabsorption of Na^+ and Cl^- is enhanced by renal tubules. Their excretion is also reduced by sweat glands, salivary glands and GIT.
2- Increased excretion of K^+ by an exchange of intracellular K^+ with extracellular Na^+.
3- Increase in extracellular fluid volume.
4- Increase in volume of circulating blood and urinary output.

Disturbance of adrenocortical functions:

Disorders of glucocorticoids:

1- Adrenal Insufficiency: (Adison's Disease)

It is characterized by hypoglycemia, extreme sensitivity to insulin, intolerance to stress, anorexia, weight loss, nausea and sever weakness.

The patients suffer from low blood pressure, decreased glomerular filtration rate and decreased ability to excrete water.

Low plasma sodium with increased potassium, eosinophils and lymphocytes.

Hyperpigmentation of skin and mucous membranes occurs due to compensatory increase in ACTH secretion.

2- Excess glucocorticoids: ***(Cushing's syndrome)***

This might be due to excessive use of steroid hormones or from ACTH secreting pitruitary adenoma.

Patient suffers from hyperglyceomia and/or glucose intolerance with severe protein catabolism. This leads to thinning of the skin, muscle wasting, osteoporosis and excessive involution of lymphoid tissue.

There is peculiar fat distribution showing truncal obesity with typical "buffalo hump".

There is impaired resistance to infection and inflammatory response with delayed wound healing.

Disorders of mineralocorticoids:

Primary aldosteronis: ***(Conn's disease)***

It is due to adenoma of glomeruolosa cells. Patients' suffer from hypertension, hypokalemia, hypernatremia and alkalosis.

C- Sex hormones:

The testes and ovaries, in addition to their function in providing spermatozoa, or ova synthesize also sex hormones.

1- Male sex hormones:

The principal male hormone, testosterone is synthesized mainly by the Leydig cells of the testes. In addition androstenedione and dehydroepiandrosterone are also produced.

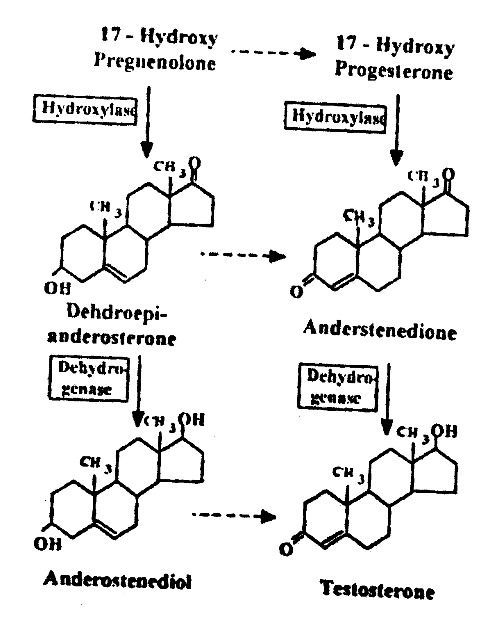
The major adrenal androgen, dehydroepiandrosterone is produced by side chain cleavage of 17-hydroxy-pregnenolone.

Metabolic actions:

1- They have protein anabolic effects, thus producing states of positive nitrogen balance. In clinical situations where promotion of protein anabolism is needed, testosterome has proved to be effective.

2- Androgens promote protein synthesis in male accessory glands, thus growth and functions of epididymis, vas deference, prostate, seminal vesicles and penis are enhanced.

- In general they are responsible for male secondary sex characters.



- Excretion of 17-ketosteroids in urine, is in part considered to be a reflection of testicular hormonal production. The test is contributes about 1/3 of total urinary 17-ketosteroids.

In normal children up to the 8th year of life there is gradual increase in 17-ketosteroids excretion with no sex difference. After puberty males show higher 17-ketosteroids excretion than females.

2- Female sex hormones:

- The two main types of female sex hormones are produced by the ovary, the follicular or oestrogenic hormones produced by cells of graffian follicles and progestational hormones derived from corpus leuteum, that is formed in the ovary from ruptured follicle.

- Small amounts of adrenal oestrogens are produced from testosterone either from dehydro-epiandrosterone or form 17-hyroxyprogesterone.

Follicular hormones:

- They are C_{18} steroids, differing from androgens in lacking methyl group at C_{10}. In contrast to all other steroids, ring A is aromatic.

- The most active hormone in circulation of all estrogens is oestradiol which is in metabolic equilibrium with oestrone.

Estriol is the principal oestrogen found in urine of pregnant women. It is produced by hydroxylation of estrone at C_{16}.

Functions:

- Follicular hormones prepare uterine mucosa for later action of progestational hormones. This include proliferation of endometrium, deepening of glands, increased vascularity etc. All these changes begin after cessation of menstrual bleeding.
- They maintain female secondary sex characters, acting antagonistic to testosterone.

Androstenedione ⇌ Testosterone

Aromatase Aromatase

CH_3 O CH_3 OH

OH OH

Estrone (E_2) Estradiol (E_1)

CH_3 OH

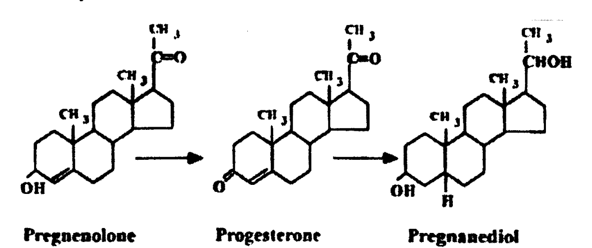
OH

OH

Estradiol (E_3)

Progesterone hormone:

Progesterone is the hormone of corpus luteum. It is also formed by adrenal cortex and placenta.



Functions:

- This homone appears after ovulation and causes extensive development of endometrium, preparing the uterus for reception of embryo, and for its nutrition.
- Progesterone also stimulates growth of the mammary glands.
- When pregnancy occurs, corpus luteum is maintained, and menstruation and ovulation are suspended. The concentration of progesterone decreases near term.
- Progesterone has antiestrogenic effect on myometrium. It decreases excitability and sensitivity of the uterus to oxytocin throughout pregnancy.

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لغة الكيمياء بين الخلايا الحية

الأستاذ الدكتور

حسين عبد المقصود على

أستاذ الكيمياء الحيوية

جامعة بنها

الباب الأول

المـــقـــدمـــــة

اللغة هي:

إحدى وسائل الاتصال الهامة التي تستخدم في تبادل المعلومـــات ، كمـــا تستخدم في تسجيل مختلف أنواع المعرفة وفي حفظها.

– وقد استخدم الإنسان منذ قديم الزمان ما يصدر عن حجرته من أصوات كوســـيلة للتخاطب والتفاهم وكأداة للتعبير عن عواطفه وآرائه ومعتقداته.

– وكان يصحبها في كثير من الأحيان بعض الإشارات باليدين أو بالرأس أو بالعينين مما قد يساعد على إيصال المعنى المقصود من فرد لآخر.

– وقد تطورت بمرور الزمن إلى كلمات محددة المعنى ، ويدل كل منها على شئ ما ، ثم تحولت بعد ذلك إلى لغة خاصة تترتب فيها هذه الكلمات بأسلوب يتناسب مع كل الظـــروف ويتمشى مع كل مناسبة.

– وقد ترتب على ذلك أن اختلفت هذه اللغات واللهجات من مجموعـــه بـــشرية إلى أخرى فتعددت هذه اللغات بين القبائل المختلفة ، واصبح لكل منها لغة خاصة بها تـــستخدمها في التعبير عن رغبتها ، وفي تبادل المنافع بين أفرادها .

– وتنوعت حتى داخل الجنس الواحد ، ويمكننا أن نرى ذلك بجلاء في القارة الهنديه ، فيوجد بها ما يزيد على تسع وعشرين لغة مستقرة ، بينما يقدر عدد هذه اللغات واللـــهجات على مستوى العالم بما يزيد على عدة مئات ، يتعامل بها البشر فيما بينهم كوســـيلة للتفـــاهم وتبادل المعرفة .

– ولا تعد اللغة المنطوقة الوسيلة الوحيدة لإجراء الاتصالات وتبادل المعلومات ، بـــل لقد ابتكر الإنسان وسائل أخرى لإجراء مثل هذه الاتصالات فاستخدم الإشارات الضوئية مثلا لتبادل الوسائل بين القطع البحرية والسفن في عرض البحار ، كما استخدم إشارات مـــورس لنقل الرسائل والأخبار عبر المسافات ، إلا أننا نلاحظ أن جميع هذه الوسائل الـــتي ابتكرهـــا

الإنسان تعتمد أساسا على حاستين هما حاستا السمع والبصر في جميع هذه الحالات ولا تخـــرج عن كونها وسائل تعتمد على إصدار الصوت أو على رؤية الصورة .

– و علينا أن نتصور أن هناك طرقا أخرى للتخاطب أو للاتصال بين أفراد الكائنـــات الحية خلاف ما نعرفه من وسائل ، كأن تفرز بعض المواد الكيميائية التى يمكن تذوقها أو شمهـــا والتعرف عليها والتأثر بها تبعا لذلك .

– وكل مادة من هذه المواد الكيميائية تستثير إحساسا خاصا عند الكائن الحى الـــذى يقوم باستقبالها ، فيصبح لكل منها بذلك مدلول خاص ، ومعنى معين ، فتشبه بذلك كلمـــات اللغة العادي ، ويمكن استخدامها بدلا من هذه الكلمات ، كما يمكن ترتيبها بأسلوب خـــاص بحيث تكون فيما بينها جملا مفيدة تحمل المعنى المقصود .

– ولو أخذنا المركبات العضوية مثالا لذلك لوجدنا أنها متنوعة التركيب وتوجد بإعداد هائلة ، فيبلغ المعروف منها حاليا ما يزيد على المليـــونين مـــن المركبـــات ، وطبقـــا لنظريـــة الاحتمالات ، يمكن أن يوجد من هذه المركبات العضوية مئات الملايين ، مما يزيد من صلاحيتها للاستخدام كمفردات في لغة الكيمياء.

– وتتميز المركبات العضوية بصفة عامة ، بأنه يمكن تغيير خواصها بإجراء تغيير طفيف في تركيب جزيئاتها ، مما يجعلها تصلح صلاحية كبيرة للاستخدام لنقل الرسائل والمعلومـــات في هذه اللغة الجديدة التى نحن بصددها .

– ولا يعتبر هذا المفهوم غريبا حقا إذا تذكرنا أن تبادل المعلومات والرســـائل داخـــل جسم الإنسان وغيره من الكائنات ، إنما يتم في الحقيقة عن طريق مثل هذه المواد الكيميائيـــة ، فالمخ يسيطر سيطرة تامة على الجسد عن طريق الرسائل الكهروكيميائيه التى يرسلها ويتلقاهـــا على الدوام وان هناك لغة كيميائية خاصة تربط بين مختلف الخلايا في الكائن الحى ، وان جميـــع الأوامر والتعليمات التى تتلقاها هذه الخلايا إنما تصدر منه واليه غلى هيئة جزيئات كيميائيـــة محددة التركيب تشبه المفردات اللغوية إلى حد كبير .

معني الحياة

– تتكون جميع الكائنات الحية من وحدات صغيرة تعرف بالخلايا ، فكل كائن حي سواء كان بكتريا أو انسانا لابد وأن يتكون من هذه الخلايا . ويمكننا أن نقدر مـدى أهميـة الخلية الحية إذا علمنا أننا نبدو على الصورة التي نرى بها أنفسنا بسبب هذه الخلايا التي تكون جسدنا ، وإذا أدركنا أن نسمة الهواء التي نستنشقها هي من عمل خلية الرئة وخليـة الـدم ، كذلك فان كل خطوة تخطوها ، وكل حركة نقوم بها ، وكل كلمة نقولها هي في الحقيقة نتـاج للتعاون بين آلاف من خلايانا العضلية والعصبية .

– وفي معرض الحديث عن الخلية الحية ، لابد وأن نتمعن قليلا في معنى الحياة ! ولو أننا سألنا أنفسنا ما هي الحياة ! لما وجدنا إجابة شافية لهذا السؤال ! ولقلنا أن الحياة هي إحدى الظواهر الرائعة في هذا الكون ، وهي تختلف كثيرا عن كل ما يحتويه هذا الكون من أعاجيب ، بل وتفوقها روعة وجمالا .

– ويصعب علينا كثيرا أن نفرق بين الشيء الحي وغير الحي إلا بعـد أن نتـدراس بعض الفروق الهامة القائمة بين كل من هذين النوعين ، *وأول هذه الفروق* أن جميع الأشـياء الحية دون استثناء تتكون من مادة معقدة التركيب تعـرف باسـم ((البروتـوبلازم)) ((protoplasm)) وهي مادة هلامية تقع داخل الخلايا ، وتتم فيها جميع الأنـشطة الحيـة والتفاعلات الحيوية .

– *والفرق الثاني* بين الأشياء وغير الحية ، هو الحساسية الشديدة للكائن الحي بمـا حوله من ظروف ... فالكائن الحي كالإنسان أو الحصان سريع التأثر عاطفيا أو سريع الغضب وسريع الاستجابة للتغيرات التي قد تحدث في البيئة المحيطة به .

- *والفرق الثالث* هو تلك الظاهرة التي نسميها بظاهرة النمو فالطفل ينمـو بعـد أن يتناول طعاما يختلف في تركيبه الكيميائي عن مادة جسده ، وهو يحول هذا الطعام عن طريـق عشرات من التفاعلات الكيميائية إلى مادة جديدة يضيفها إلى مادة جسده الأصلية .

- *والفرق الرابع* الذي يميز الأشياء الحية عن غيرها من موجودات هذا الكون هـو تلك الظاهرة المعروفة بظاهرة التكاثر . فالإنسان وأنواع الحيوان مثل القطط والكلاب لها جميعا

صغار تنمو تدريجيا حتى تتحول إلى كائنات بالغة تشبه آباءها . وحتى النباتات تعطـــي بـــذورا تتحول بدورها بمرور الوقت إلى نباتات يانعة تشبه جنسها .

– ورغم معرفتنا بكل هذه الفروق بين الأشياء الحية وغير الحية ، فما زلنا لا نعرف شيئا ما عن الحياة نفسها ، وكل ما نعرفه عنها أن الحياة ظاهرة فريدة تصاحب تلك التفاعلات الكيميائية والتغيرات الحيوية التي تحدث داخل الخلايا .

الخلية الحية

– الخلية الحية هي وحدة البناء الاساسية في الكائنات الحية دون استثناء ، وهـــي تمثل عالما قائما بذاته على درجة بالغة من التعقيد ، فتحتوي كل منها على آلاف من الجزيئات العضوية مختلفة التركيب ، كما تتم فيها مئات من التفاعلات الكيميائية المعقدة ، وتجرى فيهـــا جميع العمليات الحيوية الهامة في يسر ودقة . وقد كان العالم الإنجليزي روبـــرت هـــوك عـــام ١٦٦٥ هو أول من لاحظ وجود بعض التقسيمات المنتظمة في رقائق الفلين وأطلق عليها اسم الخلايا ((cells)) ، كذلك كان العلماء الهولنديين هم أول من وصف الخلية الحيوانية عـــام ١٦٧٣.

– والخلية الحية ذات الحجم متناه في الصغر ،فتحتوى كل بوصة مربعة مـــن جلـــد الإنسان على حوالي مليون من هذه الخلايا ، بينما يحتوى جسم الإنسان على ما يقرب من مائة تريليون خلية أو اكثر ، ويتركب مخه من حوالى ثلاثين بليون خلة

–· وتسبح الخلايا التى تكون الجسد الحي في بحر من المياه يمكن تسميته بالبحر الداخلي فهي تحتوي على ٤٠٪ من الماء الموجود بالجسم ، بينما يمثل الماء الموجود خارجهـــا حـــوالي ١٥٪ من وزن الجسم على حين يبلغ وزن الماء الموجود بالدم حوالى ٥ ٪ من وزن الجـــسم على اعتبار أن الماء يكون ٦٠ ٪ من الوزن الكلي لجسم الإنسان .

– وتأخذ الخلايا احتياجاتها من الغذاء ومن الأكسجين من السوائل المحيطة بالخلية ، كما أنها تتخلص من نواتج عملياتها الحيوية غير المرغوب فيها بإفرازها في الماء ، ومنه يلتقطها الـــدم ليحملها إلى الكليتين . وعندما يكون الكائن الحي وحيد الخلية ، أى يتكون من خلية واحـــدة مثل البكتريا ، فلا بد له أن يبحث عن وسط يحتوى على الماء كي يعيش فيه ، وآلا تعرض هذا

الكائن للجفاف والهلاك . وفي مثل هذه الحالات تقوم الخلية الواحدة المفردة بجميع الوظائف الحيوية اللازمة للكائن الحي دون استثناء ، فهي تقوم بالحركة وبالتنفس ، وبعمليات الأيض ، وبالدفاع دون أن يكون لها تخصص واضح .

– أما في الحيوانات العليا ، وفي الإنسان ، فان تخصص الخلايا يبلغ أقصى مداه ، فنجد أن هناك مجموعات متشابهة من الخلايا يتخصص كل منها في القيام بوظائف محددة لا تحيد عنها ، فهناك مثلا جهاز خاص للهضم وامتصاص الطعام ، وجهاز خاص بالتنفس وظيفته امتصاص الأكسجين والتخلص من ثاني أكسيد الكربون ، وجهاز بولي للتخلص من الفضلات الضارة بالجسم ، وجهاز للتناسل ونقل الصفات الوراثية من كائن لآخر ، وجهاز آخر يتكون من مجموعات خاصة من الخلايا تتجمع على هيئة غدد وظيفتها التنسيق الكامل بين بقية الوظائف والعمليات الأخرى ، بالإضافة إلى جهاز حاكم هو المخ .

– ونظرا لهذا التخصص الرفيع ، فانه لا يمكن القول بأن هناك خلية مثالية يمكن لها تمثل بقية الخلايا تمثيلا دقيقا ، ومع ذلك فان هناك كثيرا من الصفات المشتركة وأوجه الشبه بين كل هذه الأنواع من الخلايا .

– وتتكون الخلية بصفة عامة من جدار يعطيها شكلها العام ، ويقع بداخل هذا الجدار ذلك السائل الهلامي الذي نعرفه باسم السيتوبلازم ، ويعج هذا السائل بالنشاط الكيميائي وبالحركة الدائبة ،

– وتسبح فيه مئات من الجسيمات المختلفة الأشكال والأنواع ، فمنها الكروي ، ومنها المستطيل ، وهي تعرف بأسماء مختلفة ، مثل ((الرايبوسومات)) التي يجري عندها تشكيل جزيئات البروتينات ، و ((السنتريولات)) ، وقطرات الدهن ، و ((الميتوكوندريا)) التي توصف أحيانا بأنها محطة القوى التي تدفع بالطاقة اللازمة في أرجاء الخلية .

– ويتصف جدار الخلية بأهميته البالغة ، فهو الذي يتحكم في المواد الداخلة أو الخارجة من الخلية ، وهو يوصف عادة بأنه شبه منفذ ، أي يسمح لبعض المواد بالنفاذ منه بينما يمنع بعضها الآخر .

– ويبلغ جدار الخلية حدا فائقا من الرقة ، فيبلغ سمكه حوالي ٧٥ انجشتروم (الانجشتروم يساوى جزءا مائة مليون جزء من السنتيمتر) ، وهذا الجدار صلب إلى حد ما في الخلايا النباتية ويتكون من السيليلوز ، ولكنه في الخلايا الحيوانية يتكون من بعض الجزيئات

العضوية الكبيرة التي تترتب بجوار بعضها البعض في نظام خاص . ويتركب جدار الخلية الحيوانية من بعض الجزيئات العضوية الدهنية التي تعرف باسم ((الفوسفوليدات)) لاحتوائها على الفوسفور .

– وبالرغم من أن جدار الخلية يتكون من جزيئات متراصة لا ربط بينها ، إلا أن هذه الجدران تكفي لحفظ حدود كل خلة والاحتفاظ بشخصيتها ، فهذه الجدران تمنع انتقال السوائل بحرية مطلقة دخل جسد الكائن الحي

– وتشبه هذه الجدران تلك التقسيمات التي تقام في باطن ناقلات البترول لتقسيمها إلى عنابر صغيرة حتى لا تتحرك حمولتها من البترول بحرية مطلقة تحت تأثير أمواج البحر .

– كذلك تساعد هذه الجدران على التحكم في أنواع الجزيئات التي تدخل كل خلية أو تغادرها ، وهي بذلك تساعد بطريقة غير مباشرة على تخصص الخلايا الذي نشاهده في الحيوانات العليا وفي الإنسان .

– وللخلية الحية نواة مستديرة الشكل تقريبا تقع في وسط الخلية ويحيط بها السيتوبلازم . وتعتبر النواة من أهم مكونات الخلية الحية ، فهي المسئولة عن حياة الخلية وانقسامها وتكاثرها ، ولو أننا قسمنا هذه الخلايا الى نصفين ، لوجدنا أن النصف الخالي من النواة يفقد القدرة على الانقسام ويموت بعد فترة من الزمن ، بينما يستمر النصف الآخر المحتوي على النواة في أداء وظائفه المعتادة .

– وتحتوي النواة على أجسام صغيرة تعرف باسم الكروموسومات (الصبغيات) وهي تلك الأدوات الخاصة بحمل جميع العوامل الوراثية التي تحدد الصفات المميزة لكل كائن حي . ويتكون كل كروموسوم من غلاف من البروتين يوجد بداخله جزئ عملاق يعتبر من أهم الجزيئات العضوية التي توجد بجسم الكائن الحي ، ويعرف باسم حمض ديزوكس رايبوز النووي ، ويرمز له بالرمز DNA ((دينا)) وهو الذي يحمل الرسائل أو الوحدات الوراثية التي تعرف باسم ((الجينات))

– وتحتوي أغلب النوى على نويات صغيرة هي عبارة عن تجمعات من حمض نووي آخر يعرف باسم حمض رايبوز النووي ويرمز له بالرمز RNA وهو المسئول عن تكوين البروتينات طبقا للشفرة التي يحملها.

- وهكذا فان كل خلية في جسد الكائن الحي تعتبر عالما قائما بذاته ، له شخصيته وله وظيفته الخاصة في بعض الأحيان ، وتعج كل خلية من خلايا الكائن الحي بالنشاط والـــضجيج الكيميائي ، وتلعب كل من هذه الخلايا دورها المرسوم بكل دقة وعناية ، ويتشكل من مجموع نشاطاتها في نهاية الأمر الشكل النهائي للكائن الحي .

الباب الثانى
مفردات اللغة الكيميائية في الخلية الحية

تتعامل الخلية الحية أثناء القيام بوظائفها الحيوية مع مئات من أصناف وأنواع الجزيئات الكيميائية التى تتباين في تركيبها وفي وظائفها. وتسبح هذه الجزيئات بالسيتوبلازم والذي يملأ الخلية الحية، وهو المسئول عن كل النشاط الحيوي في جسد الكائن الحي، ففيه تحدث جميع التغيرات الكيميائية التي تؤدى الى هضم الطعام والنشاط العضلي وعمليات الأمن والدفاع وغيرها من الوظائف الحيوية.

والماء هو الوسط الذي تتم فيه كل التفاعلات الكيميائية داخل الخلية، وهو يكون حوالي ٦٠-٩٠ % من السيتوبلازم، وهو يمتلئ بأصناف متعددة من المواد الكيميائية، مثل الأملاح المعدنية، فهناك اتزان دقيق بين أيونات الكالسيوم وأيونات الصوديوم والبوتاسيوم والماغنسيوم. وتوجد هذه الأملاح في البروتوبلازم بنسبة وجودها في ماء البحر تقريبا، واستخدم بعض علماء البيولوجيا هذه الملاحظة في القول بأن الحياة بصفة عامة قد نشأت في البحار والمحيطات.

ولكل من هذه الأملاح أو الأيونات مهامه الخاصة في الخلية، فأيونات الصوديوم تساعد على ضبط عملية انتشار المواد خلال جدار الخلية، وأيونات البوتاسيوم تساعد على توصيل النبضات العصبية وعلى تقلص العضلات وأيونات الكالسيوم تسرع في عمل الإنزيمات وهكذا.

ويمكن تقسيم المواد العضوية المساندة للحياة، والتى توجد في الخلية الحية وتساهم بقدر كبير في نشاطها الحيوي، إلى ثلاثة أنواع رئيسية هي الدهون والكربوهيدرات والبروتينات. وتستحق منا هذه الأقسام الثلاث وقفة صغيرة لأنها تمثل أهم مفردات لغة الكيمياء في الجسد الحي.

أ - الدهون:

الدهون مركبات بسيطة تتكون باتحاد بعض الأحماض العضوية ذات السلاسل الطويلة مع الجليسرين. وبعض هذه الدهون لا يذوب في الماء، ولذلك تبقى منتشرة في بروتوبلازم الخلية على هيئة قطرات صغيرة الحجم. وقد ترتبط بعض هذه الدهون بذرات بعض العناصر الأخرى مثل الفوسفور والنتروجين كما سبق أن رأينا في حالة جدار الخلية الحية، وتساعد مثل هذه المجموعات على

ارتباط جزيئات الدهن بجزيئات الماء، وتجعلها أكثر قدرة على الاختلاط بما حولها من سوائل.

ب- الكربوهدرات:

الكربوهدرات مواد عضوية تتكون من عناصر الكربون والهيدروجين والأكسجين، ويوجد بها العنصران الأخيران بنسبة وجودهما في جزئ الماء وتوجد هذه المواد الكربوهدراتية على أشكال متعددة، مثل الجلوكوز (سكر العنب) والفركتوز (سكر الفاكهة)، والسكروز وهو سكر القصب الذي نستخدمه في كل بيت.

وقد تتحد بعض جزيئات الكربوهدرات البسيطة السابقة لتكون جزيئات اكبر منها لا تقبل الذوبان في الماء مثل النشا الذي يوجد في درنات بعض النباتات أو السليولوز الذى يكون جدران الخلايا النباتية ويعطي النبات شكله العام.

ج- البروتينات:

ربما كانت جزيئات البروتينات التي يجري تصنيعها في الخلية الحية من أهم مفردات اللغة الكيميائية السائدة في هذه الخلايا. والبروتينات جزيئات عضوية عملاقة تتكون من مئات من ذرات الكربون والهيدروجين والنيتروجين والأكسجين.

وأهمية البروتينات في أنها تكون أكثر من نصف المواد الصلبة التي يتكون منها جسم الإنسان. وبجانب كونها أداة هامة للحركة حيث تتكون منها أنسجة العضلات، فهي تقوم بعديد من العمليات الحيوية الأساسية، رأى تشارك في عمليات الهدم والبناء، كما أنها تتولى عمليات الدفاع الحيوي داخل جسد الكائن الحي.

الباب الثالث
المنظمات الحيوية

المنظمات الحيوية مجموعة من المواد العضوية تساهم مع الإنزيمات في تنظيم العمليات الحيوية والتفاعلات الكيميائية التي تتم في خلايا الكـــائن الحـــي. مثل الهرمونات والفيتامينات، هناك مجموعة أخرى من هذه المنظمات الحيويـــة مثل مركبات الكاينين المنظمة لأعمال جميع العضلات اللاإرادية فـــي الجـــسم، ومنظمات النمو التي تدفع خلايا البويضة المخصبة إلى الانقـــسام وتـــنظم نمـــو الكائن الحي، إلى غير ذلك من الجزيئات الكيميائية المتخصصة التي تلعب دورا هاما في حياة مختلف الكائنات.

وتلعب البروتينات دورا رئيسيا في إنتاج مثل هذه المنظمات الحيويـــة، فنجد أن لكل نوع من هذه المنظمات نوعا خاصا من الإنزيمات التي تساعد على تكوينها، أو تؤدي إلى تنشيطها، أو توقف عملها وفعلها عنـــد اللـــزوم. وهـــي كالأتي:-

١- الإنزيمات:

الإنزيمات إحدى المفردات الهامة في لغة الكيمياء داخل جـــسد الكـــائن الحي، تساعد على ترجمة الأوامر الصادرة في الخلية إلى واقع ملمـــوس وهـــي المسئولة عن كل عمليات الهدم والبناء التي تقوم بها الأجسام الحية.

الطريقة التي تنجز بها الخلية تفاعلاتها الكيميائية في يسر ودقة إحـــدى الظواهر التي طالما أثارت إعجاب علماء الكيمياء. فمن المعروف أن مثل هـــذه التفاعلات المعقدة لا تحدث خارج الخلايا في المعامل إلا في بطء شديد، وتحتاج إلى طاقة عالية تستلزم رفع درجة الحرارة إلى حدود تزيد كثيرا علـــى درجـــة الحرارة السائدة في هذه الخلايا.

وقد اتضح فيما بعد أن الخلايا الحية لا تحتاج إلى كل هذا الجهد لإتمـــام عملياتها الحيوية، فهي تقوم بتصنيع أدواتها الخاصة المعروفة باسم الإنزيمـــات، وهي التي تعمل كعوامل مساعدة وتنجز كل أعمالها في يسر ودقـــة. والعامـــل المساعد مادة نشيطة تساعد على دفع التفاعل الكيميائي إلى نهايتـــه، ولكنهـــا لا تدخل في هذا التفاعل بل تبقى ثابتة دون تغير عند نهاية هذا التفاعل.

وقد كان العالم البريطاني الكسندر فليمنج هو أول من اكتشف أحد هـــذه الإنزيمات فقد كان مصابا بنوبة برد، وهداه تفكيره العلمي إلى أن يضع بعـــض قطرات من السائل المخاطي الذي يتساقط من انفه في طبق من الزجاج يحتـــوي

على مزرعة من البكتريا، ثم وضع هذا الطبق جانبا فترة من الزمان كي يـرى نتيجة هذه التجربة. وقد دهش فليمنج عندما لاحظ أن البكتريا المحيطة بقطـرات السائل المخاطي قد بدأت في التحلل والذوبان بمرور الوقت، وتصور في الحـال أنه على وشك اكتشاف مضاد حيوي جديد يستطيع إبادة كل أنواع البكتريا علـى الإطلاق.

وقد خاب ظن فليمنج بعد أن علم أن الفعل المضاد للبكتريـا كـان فـي الحقيقة نتيجة لوجود أحد الإنزيمات في المخاط، وان لهذا الإنزيم قدرة على إذابة وتحليل خلايا البكتريا وسمي فيما بعد (لايسوزايم) أو الإنزيم المحلل. وقد أدرك فليمنج أن هذا الإنزيم لا يصلح لإبادة كل أنواع البكتريا، فقضى حـوالي سـبع سنوات من البحث قبل أن يتوصل لاكتشاف المضاد الحيوي الهام الذي عـرف فيما بعد باسم البنسلين.

٢- مركبات الكاينين:

مركبات الكاينين هي احدى مفردات اللغة الكيميائية السائدة في أنـسجة الكائنات الحية، ولا تفرز مركبات الكاينين من غدد خاصـة كمـا فـي حالـة الهرمونات، ولكنها تتكون في أغلب الأحوال في الأماكن التي تحتاج الى فعلهـا الكيميائي. وهي لا تعيش طويلا، فهي سرعان ما تتفكك وتتحلل بعد أن ينتهـي عملها الحيوي. وقد أطلق اسم الكاينين على هذه المركبات لأنها تـسبب تقلـص العضلات، وانبساط الأمعاء وانقباضها، ولهذا سـميت بمركبـات الكـاينين أي المركبات المسببة للحركة.

وتنتمي هذه المركبات إلى مجموعة البروتينات، فهي تتكون من تتـابع من وحدات الأحماض الأمينية، ولكن الجزيئات في هذه الحالة تكـون صـغيرة نسبيا.

وقد اكتشفت مركبات الكاينين أول الأمر في أثناء إجراء بعض التجارب الفسيولوجية على قطع من الأمعاء الدقيقة للإنسان. فعند تعليق قطعة مـن هـذه الأمعاء أخذت عقب الوفاة، في محلول يماثل الدم في ملوحته وقلويته، ويمر بـه تيار من الأكسجين أخذت هذه القطعة في الانقباض والتمدد تلقائيا فـي حركـة منتظمة يمكن ملاحظتها بالعين المجردة. ولم يستطع أحد أن يجد تفسيرا مقبـولا لهذه الظاهرة حتى عام ١٩٣٧ عندما قام بعض العلماء الألمان بإجراء تجربـة فريدة في هذا الشأن أدت إلى اكتشاف الطريقة التي تعمل بها مركبات الكاينين.

ويحتوي جسد الإنسان على ميكانيكية فائقة الدقة، تحقق له نوعا ثابتا من الاتزان على الدوام. فعندما تكون هناك بالجسد عملية ما تؤدي إلى إطلاق أحـد العوامل العالية النشاط، نجد أن هناك عملية أخرى تطلق عاملا آخـر يـستطيع إيقاف فعل هذا العامل النشيط، إما بتحويله إلى مادة خاملة لا أثـر لهـا، وإمـا بتحليله تحليلا نهائيا، وإلا انقلب هذا العامل النشيط إلى شئ شره مدمر يكتسح كل ما يقابله.

ولا تؤثر مركبات الكاينين في جميع عضلات الجـسم، ولكـن أغلـب العضلات التي تستجيب لهذه المركبات هي من النوع المعروف باسم العـضلات ألا إرادية، مثل العضلات التي تتحكم في الأجزاء المجوفة مـن الجـسم مثـل الأمعاء والأوردة والشرايين، وقنوات القصبة الهوائية في الرئتين والقنوات التي يجري فيها البول. كذلك تؤدي بعض هذه المركبات إلى نفاذ الدم خلال جـدران الشعيرات الدموية وبذلك يستخدمها الجسم في دفع الدم في الأماكن التـي تعمـل بصفة دائمة أو تعمل بكثرة، مثل عضلات جدران الأمعاء وبعض الغـدد التـي تعمل بشكل متواصل.

ومن العجيب أن كل ما تستطيع أن تقوم به مركبات الكاينين، يستطيع أن يقوم به تقريبا مركب آخر يعرف باسم (الهستامين) والذي يفرزه الجـسم عنـد الضرورة. ويبدو أن جسد الكائن الحي لديه أكثر من وسيلة لبلوغ نفس الهـدف، وفي مثل هذه الموقف لا يمكن القطع بأن أحدهما أكثر أهمية من الأخر.

فانه من الواضح أن مركبات الكاينين أنها المحرك الحقيقي وراء ذلـك العمل الدائب الذي تقوم به العضلات اللاإرادية من ساعة إلى أخـرى، وبـذلك تعتبر مركبات الكاينين إحدى المفردات الهامة في هذه اللغة الكيميائية التي نحن بصدد الحديث عنها.

٣- الهرمونات:

تنتمي الهرمونات الى مجموعة المنظمات الحيوية، وهي تلعب دورا هاما في بعض العمليات الحيوية التي تجري في جسد الكائن الحي.

والهرمونات عبارة عن جزيئات بروتينية صغيرة الحجم نسبيا، وهي لا توجد في كل مكان في جسد الكائن الحي، ولكنها تفرز بواسطة أنواع خاصة من الخلايا التي تكون معا غددا خاصة لكلُ نوع من هذه الهرمونات، وهي تنطلـق بعد ذلك في الدم الذي ينقلها إلى المواقع المطلوبة فيها أو تطلق مباشرة في مكان

عملها. ولا تفرز الهرمونات من هذه الغدد بصفة مستمرة، ولكنها تفــرز تحــت بعض الظروف أو استجابة لبعض المؤثرات الخاصة.

وأهم ما يميز الهرمونات نوعيتها الفائقة، أي أن لكل منها أثرا محــددا، ووظيفة ثابتة لايتعداها أبدا، فالهرمون الواحد قد يؤثر تأثيرا خاصا في نوع مــا من الخلايا، فيدفعها إلى الدخول في تفاعلات بعينها، ولكنه لا يؤثر بتاتــا علــى بقية الخلايا الأخرى المحيطة بها، فتستمر هذه الخلايا في عملها المعتاد وكأنها لا تحس به على الإطلاق، وكأن كلا منها له لغته الخاصة به. ومــن أمثلــة هــذه الهرمونات، هرمون الأنسولين الذي يفرزه البنكرياس، والذي يتحكم في عمليات التمثيل الغذائي للسكريات.

ومن أهم أنواع الهرمونات التي تحتويها أجساد الكائنات الحيــة تلــك الهرمونات المعروفة باسم هرمون الجنس، وهي تلك المواد التي تعطي كل جنس صفاته المميزة، وتساعد بذلك على التفرقة بــين الــذكر والأنثــى. ولا تنتمــى هرمونات الجنس إلى مجموعة البروتينات، ولكنها تنتمي من الناحية التركيبيــة إلى مجموعة أخرى مــن المركبــات العــضوية تعــرف باســم (ســتيرويدات steroids).

٤ – الفيتامينات:

الفيتامينات من أهم أفراد مجموعة المنظمات الحيوية، فهي تلعــب دورا هاما في تنظيم العمليات الحيوية في جسد الكائن الحي، ويؤدي النقص فيها فــي كثير من الحالات الى اختلال نظام الجسد والمرض وينتهي الأمر بالوفاة. وقــد كان البحارة هم أول من قاسى من نقص الفيتامينات، وذلك لأن رحلاتهم الطويلة في البحر كانت تستدعي بقاءهم فترة طويلة بين الماء والسماء، ولم يكن غذاؤهم وطعامهم متنوعا بشكل كاف.

ويذكر التاريخ أن بعض هؤلاء البحارة كانوا يمرضون سبب معــروف، وكان المرض ينتهي في أغلب الحالات بالوفاة، وقيل في ذلك الوقت أن البحارة يصابون بمرض خاص سمي الإسقربوط.

وتروي لنا الكتب أن الرحالة الشهير (فاسكودي جاما) فقد حوالي مائــة رجل من رجاله بسبب هذا المرض أثناء رحلته الطويلــة حــول رأس الرجــاء الصالح. كذلك تروي قصص مماثلة عن بحارة الرحالة (ماجلان). فقــد تــساقط منهم عدد كبير صرعى دون سبب معروف، وان كان يبدو أن هناك عاملا هاما كان ينقص غذاء هؤلاء البحارة في رحلاتهم الطويلة.

وقد اكتشف البحارة الإنجليز قدرا أنهم لا يصابون بهذا المرض الغريب أبدا إذا تناولوا بعضا من عصير الليمون على فترات أثناء رحلاتهم الطويلة. وأصبح تناول هذا العصير شيئا مقدسا على السفن البريطانية منذ ذلك الحين.

ولا تتشابه الفيتامينات في تركيبها، بل هي تختلف فيما بينها اختلافا كبيرا، فمنها ما تحتوى جزيئاته على الكربون والهيدروجين، ومنها ما يحتوي على النيتروجين، ومنها كذلك ما يوصف بأنه حمض أو كحول، كما أن منها ما ينتسب الى مجموعة السيترويدات مثل فيتامين (د). ولا شك أن هذا الخلاف في التركيب بين الفيتامينات، هو الذي جعل لكل منها معنى خاصا في لغة الكيمياء في أجساد الكائنات الحية، وجعل لكل منها وظيفة بعينها في العمليات الحيوية التي تتم داخل الخلية الحية.

وقد لوحظ أن بعض هذه الفيتامينات قد تصلح كفيتامين في إحدى فصائل الثدييات، ولكنها لا تصلح لذلك في فصيلة أخرى، ويرجع ذلك أساسا إلى أن جميع الكائنات الحية لا تتكلم نفس اللغة ولا تستخدم نفس المصطلحات الكيميائية، بل توجد هناك بعض الفروق الطفيفة في كيمياء التمثيل الغذائي بين هذه الطوائف. ولأن الفيتامينات لا تشترك إلا في مرحلة معينة من عمليات التمثيل الغذائي، فان غياب الفيتامين عن الخلية الحية يتسبب في توقف هذه المرحلة، مما قد يؤدي كذلك إلى توقف ما يتلوها من مراحل، ولابد أن ينتج عن ذلك قصور ما في عمليات التمثيل الغذائي تظهر آثاره فيما بعد على هيئة أعراض المرض.

ونظرا لأهمية الفيتامينات لجسد الكائن الحي فقد ظن البعض أن استخدام كميات كبيرة من هذه الفيتامينات قد يشفى بعض الامراض خاصة تلك الأمراض الخاصة بالجهاز العصبي، ولكن تبين بعد ذلك أن الجسم لا يستخدم منها الا ذلك القدر الضئيل الذي ذكرناه أمام كل منها من قبل، ولا يستطيع الاستفادة من الكميات الزائدة منها.

وتؤدي الزيادة في تناول الفيتامينات إلى الإضرار بصحة الفرد وإصابته ببعض العلل والأمراض، ومثال ذلك ظهور بعض أعراض التسمم عند تناول جرعات كبيرة من فيتامين (أ) أو فيتامين (د)، أو فيتامين (ك)، وكثيرا ما يصحب هذه الأعراض تضخم الطحال وسقوط الشعر وآلام العظام.

ويتضح أن هناك توازنا دقيقا يُجب المحافظة عليه ضمانا لصحة جسد الكائن الحي، فنقص الفيتامينات يؤدي إلى الإصابة بعديد من الأمراض، وزيادتها عن الحد المطلوب يؤدي كذلك إلى الإصابة بغيرها من الأمراض. أما الفائدة

الحقيقية من هذه الفيتامينات فتكون عند حصول الجسد على القدر المناسب منهـا والذي يكفي فقط لاستكمال العمليات الحيوية التي تجري داخل الخلية الحية.

ويمكننا الآن أن نشتري هذه الفيتامينات من الصيدليات إذا أردنا ذلك أو إذا كان غذاؤنا لسبب من الأسباب لا يحتوي على الكفاية منهـا، إلا أنـه مـن الأفضل دائما أن نعتمد على الغذاء الطبيعي كمصدر للفيتامينات لأسباب متعـددة أهمها أن الغذاء الطبيعي قد يحتوي على أكثر من فيتامين واحد، بجانب الأملاح والأحماض العضوية وغيرها من المواد الهامة للجسم. وبـالرغم مـن أن كـل الفيتامينات يرمز لها بحروف الهجاء، إلا أن هناك اتجاها حديثا لتسميتها بأسماء خاصة، فيعرق الآن فيتامين ب١ باسم الثيامن، وفيتامين ب٢ باسم ريبـوفلافين وهكذا.

ولا يحتاج جسد الكائن الحي إلا لقدر ضئيل جدا من الفيتامينات، وهي لا تدخل في كل العمليات الحيوية بالخلية الحية، ولكن كل منها يتخصص في عمل ما لا يحيد عنه. وتقدر كمية الفيتامين التي يحتاج إليها الجسم بعدة وحدات، فهي أما أن تقاس بالمليجرامات (جزء من ألف جزء من الجـرام) أو بـالميكروجرام (جزء من مليون جزء من الجرام) أو بالوحدات الدولية التي يساوي كـل منهـا ١/٤٠ من الميكروجرام.

أ- فيتامين أ:

يتركب جزئ فيتامين (أ) من عشرين ذرة من ذرات الكربون وهو لنمو الجسم نموا صحيحا، كما انه هام جدا بالنسبة لسلامة وصحة الخلايـا المكونـة للجلد والخلايا المغطية لكل تجاويف الجسم من الداخل. ويعاون فيتامن (أ) العين على استقبال الضوء، ويتسبب النقص فيه في عدم قدرة المرء على الرؤية الجيدة ليلا، فسائق السيارة قد يبهره ضوء السيارة المقبلة من الناحيـة الأخـرى مـن الطريق ليلا، ولكنه يستطيع أن يرى الطريق جيدا بعد أن تمر هذه العربة. أمـا إذا كان هذا السائق مصابا بنقص في فيتامين (أ) فانه لن يستطيع أن يرى أمامه إلا عدة أمتار من الطريق فقط بعد أن تمر السيارة الأخرى وتعرف هذه الظاهرة بالعشى الليلي.

ويوجد فيتامين (أ) في زيت كبد الحوت وفـي الزبـد والجـبن واللـبن والقشدة، كما يوجد على هيئة مركب الكاروتين الذي يتحول إلى فيتامين (أ) في الجسم، في كثير من الخضراوات وفي الجزر والسبانخ والطماطم، وفي المشمش

والخوخ والموز. وغالبا ما يكون النقص في فيتامين (أ) ناتجا عن عــدم قــدرة الجسد على امتصاص الفيتامين، وذلك بسبب المرض أو غيره من الأسباب.

ب- فيتامين ب المركب:

عبارة عن مجموعة من الفيتامينات التي تقبل الذوبان في الماء. وتختلف فيتامينات هذه المجموعة في التركيب الكيميائي، ولكنها تتشابه في وظائفها فــي الجسد الحي، وتوجد معا في كثير من المواد الغذائية. ويقع تحت هذه المجموعة، الثيامين (فيتامين ب١) والرايبوفلافين (ب٢) والنياســين (حمــض النيكــوتين) والبيريدوكسين (ب٦)، وحمــض بانتوثينيــك، وبيــوتين، وحمــض الفوليــك، وسبانوكوبالامين (ب١٢).

١- الثيامين (فيتامين ب١):

يوجد مختزنا في الكبد، ويساعد على النمو السليم، وعلى سلامة عمــل الأنسجة العصبية. وينتج عن نقص هذا الفيتامين مرض البــري بــري. ومــن مظاهره الشعور بالإرهاق، وفقدان الشهية، واضطراب الهضم، والهــزال،وقــد ينتهي بالإصابة بالشلل والوفاة. ويوجد هذا الفيتامين في بعض البقول مثل البسلة والفاصوليا،والفول السوداني، وتعتبر قشور الأرز وقشور القمح (الردة) مــن المصادر الغنية بالثيامين.

وينتشر النقص في هذا الفيتامين بين سكان بلاد الــشرق الأقــصى، لأن غذائهم الرئيسي يتكون من الأرز الذي نزعت منه القشور.

٢- الرايبوفلافين (فيتامين ب٢):

تكمن أهمية هذا الفيتامين في أنه يكون جزءا هاما من أحــد الإنزيمــات التي توجد في كل خلايا جسم الإنسان، والذي له علاقة كبيرة بتــنفس الخلايــا. ومصادر هذا الفيتامين متعددة، فهو يوجد في الكبد، وفي البيض وفــي البقــول والطماطم وبعض أنواع الفاكهة. ويختزن الرايبوفلافين في الكبد وفــي الكليــة، ويتسبب النقص فيه، في ضعف نمو الأطفال، وتشقق الجلد حول الفــم، وتــورم اللسان، وازدواج الرؤية، كما يتسبب في تعرض الجلد لبعض الأمراض الجلدية الضارة. والكمية الأزمة لصحة الجسم ١,٧ مليجرام في اليوم.

٣- النياسين :

يعرف كذلك باسم حمض النيكوتين. ويدخل هذا الفيتـامين فـي نظـام الإنزيمات التي تنقل الهيدروجين في الخلايا الحية وهي عملية هامة من عمليات النشاط الحيوي في الخلية. ويؤدي نقص النياسين بالجسم إلى مرض البلاجـرا، وهو ينتشر بين سكان البلاد الذين يعيشون على الدقيق الأبيض والأرز المبيض. وينتج عن ذلك احمرار الجلد، ونقص في وزن الجسم، وحدوث إسهال وظهـور الاضطرابات العقلية على المريض. وقد يؤدي عدم علاج المريض إلى حـدوث الجنون ثم الوفاة.

ويوجد النياسين في اللحم الأحمر والكبد والخميرة واللبن والبيض وفـي بعض البقول. كذلك يمكن الحصول عليه من السمك، فكل ٢٥٠ جم من الـسمك تحتوي على ٤٥٠ مليجرام من الحمض الاميني (التربثوفان) ويستطيع الجسم أن يحول هذه المادة الأخيرة ال ٧,٥ مليجرام من النياسين، ولهذا السبب فـان مـن يتغذون على الأسماك لا يعانون نقصا في الفيتامين على الإطلاق.

٤-_البيريدوكسين فيتامين (ب٦):

يشبه هذا الفيتامين في عمله بقية أعضاء مجموعة فيتامين (ب) المركب، فهو هام لصحة الجلد، ولسلامة النشاط العصبي للكائن الحي، كما أنه له أهميـة خاصة في عمليات تمثيل الأحماض الأمينية في الجـسم وتكـوين البروتينـات وغيرها. ويوجد البيريدوكسين مع غيره من أعضاء هذه المجموعة فـي الكبـد والبيض والخميرة وبعض الأغذية المعتادة.

٥- حمض البانتوثنيك :

أحد أعضاء مجموعة فيتامين (ب) المركب، وهو يوجـد فـي جميـع الأنسجة الحية، ولهذا أطلق عليه كلمة (بانتوثين pantothen) (التي تعني (فـي كل مكان). وتكمن أهمية هذا الفيتامين في أنه يكون جزءا من أحد الأنزيمـات ويعرف باسم (مساعد الإنزيم أ co-enzyme A)وهو يدخل في عديد من التفاعلات الحيوية في الخلية الحية. ويوجد حمض البانتوثنيك في الكبد والخميرة والبيض وفي اللحوم ومنتجات الألبان، وفي العسل الأسود والفاكهـة، كـم أنـه يصنع في الأمعاء بواسطة البكتيريا، ولهذا يندر أن يصاب الفرد العادي بـنقص في هذا الفيتامين.

ويعد غذاء ملكات النحل المعروف باسم (الغذاء الملكي) من أهم مصادر هذا الفيتامين. ويؤدي النقص في هذا الفيتامين إلى نقص النمو، والإحساس بالصداع والشعور بالغثيان، وتغير لون الشعر، كما يؤدي أحيانا إلى الشلل ثم الوفاة.

٦- البيوتين:

أحد أعضاء مجموعة فيتامين (ب) المركب، وسمي فترة بفيتامين (هـ H)، ويوجد هذا الفيتامين في كثير من الأطعمة كما يتكون في الأمعاء بواسطة بعض أنواع البكتريا. وقد تبين أن بعض الحيوانات أو الإنسان إذا تغذت على زلال البيض النيئ فترة طويلة نتجت عن ذلك أضرار كثيرة، منها نقص الوزن وفقد استقامة الجسم وغير ذلك من الأعراض. وقد استطاع البيوتين أن يعالج هذه الأعراض، ولذلك سمي العامل المضاد للزلال البيض. ولا يحتاج الجسم من هذا الفيتامين إلا إلى قدر ضئيل جدا، ولهذا يندر أن يشعر الإنسان بنقص الفيتامين.

٧- حمض الفوليك:

أحد أعضاء مجموعة فيتامين (ب) المركب، وهو حمض مشتق من حمض الجلوتاميك. وقد سمي هذا الفيتامين بذلك الاسم لأنه اكتشف أول مرة في بعض الخضراوات ذات الأوراق الخضراء، حيث كلمة (فوليوم) (folium) اللاتينية تعني ورق الشجر. ويؤدي النقص في هذا الفيتامين إلى فشل نخاع العظام في تكوين كريات الدم الحمراء مما يؤدي إلى الإصابة بالأنيميا.

ويوجد حمض الفوليك في الفواكه الطازجة وفي الكبد والكلية وفي الخميرة.وهو يعطي للمصابين بالحروق أو بالإشعاع، أو المصابين بكسور في العظام، وكذلك للمصابين بالأنيميا، وعادة ما يضاف اليه فيتامين (ب١٢) في الحالة الأخيرة.

٨- فيتامين ب١٢:

مركب عضوي يحتوي على فلز الكوبالت في جزئيه، ويعرف باسم سيانوكوبالامين. وقد تبين أن قدرا ضئيلا من هذا الفيتامين، يكفي لعلاج مرض الأنيميا الخبيثة، وهذا القدر لا يزيد على جزء من مليون جزء من الجرام، أي ميكروجرام، واحد في اليوم.

ج- فيتامين (ج):

حمض الاسكوربيك. ويشبه هذا الفيتامين في تركيبه السكر (الكربوهدرات)، وهو يلعب دورا هاما في تكوين الأنسجة الضامة في الجسم وأنسجة العظام والأسنان وسلامة جدران الأوعية الدموية في الجسم. ويختزن هذا الفيتامين في الجسم في بعض الغدد، مثل الغدة الكظرية، وكذلك في الكبد والكلية وبعض أجزاء الجسم الأخرى. وهو يطلق في الجسم أثناء المجهود العضلي غير العادي أو أثناء التوتر العصبي الشديد.

وينتج عن نقص فيتامين (ج) الإصابة بمرض الإسقربوط حيث تتورم المفاصل، وتسبب آلاما مبرحة، وتتورم اللثة وتصبح الأسنان غير ثابتة، ويصحب ذلك حدوث نزيف تحت الجلد. ويوجد فيتامين (ج) في كثير من الخضراوات ذات الأوراق الخضراء، وفي الفاكهة، خاصة الموالح، كما يوجد في الكرنب والفلفل الأخضر.

وتقوم كثير من الحيوانات بتكوين هذا الفيتامين في جسدها، ولكن الإنسان وبعض الحيوانات الأخرى مثل القردة وفئران التجارب وبعض أنواع البكتريا، لاتفعل ذلك، ولا بد لها من الحصول عليه عن طريق الغذاء من الخارج.

ويتأثر فيتامين (ج) بالحرارة، فهو ينحل بالتسخين، ولهذا لابد من حفظ الفاكهة والخضراوات في الثلاجات عند تخزينها حتى لا تفقد ما بها من فيتامين (ج). وينحل جزء كبير من فيتامين (ج) عند طهي الطعام، خاصة عندما يتم ذلك في أوعية مفتوحة معرضة لأكسجين الهواء. ويمكن الاحتفاظ بجزء كبير من هذا الفيتامين كما هو، عند استخدام أوعية الضغط في طهي الطعام وذلك لأن تسخين الطعام في هذه الحالة يتم بعيدا عن أكسجين الهواء.

وقد ظن بعض العلماء أن تناول جرام واحد من هذا الفيتامين كل يوم، يمنع إصابة الفرد بالبرد ونزلات الزكام، ويقلل من فرص المرض بصفة عامة، وأن تناول جرعة كبيرة تبلغ عشرة جرامات في اليوم، وقد تحسن كثيرا من حالة المرضى بالسرطان، ولكن التجارب العلمية المتأنية التي أجريت في هذا المجال دلت على أن فيتامين (ج) ليست له فوائد علاجية لهذه الأمراض.

د- فيتامين (د):

عبارة عن مجموعة من المواد التي تقبل الذوبان في الدهون، ومنها فيتامين (د٢) (ارجوكالسيفيرول)، وفيتامين (د٣) (كوليكالسيفيرول). وتوجد

مجموعة فيتامين (د) في زيت كبد الحوت، وفي بعض النباتات التي تحتوي على مواد يمكن أن تتحول إلى هذا الفيتامين تحت تأثير الأشعة فوق البنفسجية، والتي تسمى عادة بمشابهات الفيتامينات (provitamins)، مثل الارجوستيرول، ونوع من مركبات الكولسترول.

ويعتبر فيتامين (د) مضادا للكساح عند الأطفال، فهو يحافظ على توازن أيونات الكلسيوم والفوسفور في الجسم، ويتسبب النقص فيه في حدوث الحالة التي نعرفها باسم لين العظام، وذلك لتأثر فوسفات الكلسيوم الموجودة بالعظام التي لا تتحمل وزن الجسم فتنحني تحت ثقله وتتقوس. وينتج كذلك عن هذا النقص كبر حجم المفاصل، وعدم انتظام الأسنان وتغير شكل القفص الصدري، وضعف العضلات العام.

ويتأثر الكبار كذلك بنقص هذا الفيتامين، فيؤدي النقص فيه إلى سحب فوسفات الكالسيوم من العظام، مما يجعلها أكثر مسامية وأكثر عرضة للكسر. ويمكن الحصول على فيتامين (د) يوميا بتعريض الجسم للأشعة فوق البنفسجية لمدة معقولة، حيث تتحول بعض مشابهات الفيتامينات الموجودة تحت الجلد إلى هذا الفيتامين، ولهذا يندر أن يوجد مرض الكساح في البلاد المشمسة. والزيادة الكبيرة في كمية فيتامين (د) ذات أثر سيئ على صحة الإنسان، فهي تؤدي إلى سحب الأملاح من العظام، وزيادة الكلسيوم في الدم وتكوين الحصى في الكليتين، وترسب الكلسيوم في الأنسجة اللينة.

هـ- فيتامين ك:

يعرف باسم فللوكينون، وهو مضاد للنزيف ويسبب تخثر الدم. وقد تم اكتشاف مشابه له سمي فارنوكينون (فيتامين $ك_٢$) عام ١٩٤٣، وينتج الآن في السوق العالمي مركب تخليقي باسم (مناديون) له نفس نشاط وفعل هذا الفيتامين.وتوجد كميات مناسبة من هذا الفيتامين في السبانخ، وفي زيت الصويا، والطماطم وقشور الأرز كما يتم تكوينه في الجسم بواسطة نوع من البكتريا الموجودة بالأمعاء. ويعتبر هذا الفيتامين مسئولا عن تكوين مادة البروثرومبين في الكبد وهي التي تؤدي إلى تجلط الدم.

ويندر أن يتعرض الشخص البالغ لنقص هذا الفيتامين لوجود البكتريا التي تكون هذا الأنزيم في أمعائه. أما الأطفال حديثو الولادة، فلا توجد في أمعائهم هذه البكتريا كما أن كمية الفيتامين التي يحصل عليها الطفل من جسد أمه

قليلة جدا، ولهذا يتعرض الأطفال حديثوا الولادة في بعض الأحيان لأنواع مـــن النزيف، ويمكن التغلب على ذلك باعطاء الوليد جرعة صغيرة من فيتامين ك.

و- فيتامين هـــ E:

عبارة عن مجموعة من المركبات متـــشابهة التركيـــب تعـــرف باســـم (توكوفيرول) (Tocopherols). ويسمى هذا الفيتامين بالفيتامين المضاد للعقم، حيث أن النقص فيه يؤدي إلى حالات من العقم عند الفئران، وعدم وصول الـــدم إلى العضلات. ولا توجد هناك أدلة على أن هناك نقصا ما في هذا الفيتامين عند الإنسان.

الباب الرابع
مفهوم اللغة الكيميائية

ويمكن تصور مفهوم اللغة الكيميائية بطريقة أفـضل إذا انتقلنـا إلـى مملكة الحشرات، من المعروف أن هذه الحشرات تعيش في تجمعات خاصة تشبه المجتمعات البشرية، تتم فيها الأعمال بمنتهى الدقة والنظام.

وتبين أن بعض الحشرات تقوم بإفراز بعـض المـواد الكيميائيـة فـي مناسبات معينة، وهى تفعل ذلك أما للتأثير المباشر في الأفراد المحيطة بها، وإما للتحكم في البيئة نفسها، وهى تقوم بذلك ببراعة، رأى لا تخلـط بـين مناسـبة وأخرى ولا تخطئ في ذلك على الإطلاق، فلكل مناسبة مادة خاصة بها، كما أن لكل مادة غدة خاصة بها أيضا.

وتبدو هذه الظاهرة بوضوح في مستعمرات النمل الأبيض، فان كلا من جماعات النمل المتخصصة للتكاثر، وجماعات الجنود التى تتولى الـدفاع عـن المستعمرة، تفرز مادة كيميائية معينة تمنع جماعات النمل الأخرى من التحـول إلى صورتها. وبذلك يتم الاحتفاظ بالتوازن فـي أعـداد كـل صـنف داخـل المستعمرة. هذه المواد الكيميائية تؤثر على الغدد الصماء في هذه الكائنات،

لا يقتصر الأمر على مستعمرات النمل فقط، بل تنتشر هذه الظاهرة بين غيرها من أفراد مملكة الحشرات، فذكور الجراد البالغة تقوم بإفراز مادة كيميائية متطايرة من السطح الخارجى لجلودها لتساعد على الإسراع في نمو أفراد الجراد الصغير السن. وقد اتضح أن عذراء الجراد عندما تحس بوجود هـذه المـادة، ترتجف قرون الاستشعار بوضوح كما أن أرجلها الخلفية وبعض أجـزاء فمهـا تهتز عند تعرضها لهذه الرائحة.

كذلك تمتد هذه الظاهرة لتشمل كثيرا من أفراد مملكـة الحيـوان، وقـد اتضح كذلك أن رائحة ذكور الفئران تساعد على بدء دورة المبيض فـي إنـاث الفئران، كما أن رائحة ذكر فار غريب قد تتسبب في إيقاف حمل حديث لإحدى الإناث، بينما نلاحظ أن رائحة الذكر الأصلي الذى تسبب في الحمل لا اثر لهـا طبعا على حمل هذه الأنثى.

ويعتقد بعض العلماء أن هناك بعضا من الأدلة على أن رائحة ذكر الفار الغريب تتسبب في تعويق إفراز هرمون (البرولاكتين) مما يـؤدى إلـى عـدم استكمال نمو إحدى غدد المبيض عند أنثى الفأر.

وتدل هذه الظواهر جميعا على أن هناك لغة خاصة بين هـذه الكائنـات الحية تستخدم فيها بعض المركبات الكيميائية التى تقوم بإفراز كل منها في إحدى المناسبات الخاصة أو من اجل غرض معين .

وقد تم فصل بعض المواد الكيميائية البسيطة التى ينتشر استخدامها في مملكة الحيوان لإحداث اثر واحد محدد، وتم التعرف على تركيب بعض منهـا. وتحدث مثل هذه المواد تأثيرا مباشرا على الجهاز العصبي المركـزي، وتخـدم بذلك كثيرا من الأغراض والوظائف، فهي قد تحدد سلوك الحيوان بالتأثير فـي تصرفاته المباشرة، ويمكن بذلك اعتبارها بديلا للغة الكلام فهي تسهم في تبـادل المعلومات وفى تلقى الأوامر المباشرة، ومن أمثلة هـذه المـواد تلـك المـواد المعروفة باسم (مواد الأثر) وهى المواد التى يستخدمها النمل لتحديد اتجاهـات سيره وحركاته خارج المستعمرة، و(مواد الإنذار) وهى المواد التـى تطلقهـا الحشرات للإنذار بوقوع الخطر،و((جاذبات الجنس)التى تطلقها الإنـاث لجـذب ذكور الحشرات.

ويتضح مما سبق أن لكل مادة كيميائية أثرا معينا، وفعلا خاصا تنفرد به هذه المادة دون غيرها، وانه إذا أطلقت هذه المادة بين كائنين أشبهت فـي ذلـك الكلمات والجمل المفيدة التى تتكون منها لغة الكلام عند الإنسان.

أما إذا أطلقت هذه المواد داخل جسد الكائن الحى، فإنها تشبه فـي ذلـك الرسائل المكتوبة التى تحمل التعليمات، وتحدد خط السير وأسلوب العمل، وهى تماثل في ذلك تعليمات التشغيل أو الشفرة، وهذه هي الطريقة التى تعمـل بهـا الأحماض النووية داخل أجساد الكائنات الحية، كما تفعل ذلك كثير مـن المـواد الكيميائية الأخرى، مثل مركبات الكاينين والإنزيمات ومنظمات النمو وغيرها.

مـواد الأثـر

ربما كانت اكثر أنظمة الاتصال الكيميائية تطورا فهي الطبيعية، هي تلك الأنظمة التى تستخدمها بعض أفراد مملكة الحشرات الفائقة التنظيم مثل النحل أو النمل.

لو فحصنا مساكن هذه الحشرات، لوجدنا أن كلا من المستعمرات التـى يقيمها النمل، والخلايا التى يبنيها النحل تتكون من سـراديب متـشعبة يغـشاها الظلام الدامس على الدوام، وبذلك فان أفراد هذه الأنواع من الحـشرات تعـيش عادة في ظلام تام داخل هذه الخلايا أو المستعمرات، حتى أنه قد يصعب علـى إحداها أن تميز الأخرى داخل ممرات ودهاليز هذه المساكن المتشعبة.

وينبنى على ذلك أن أفراد هذه المجموعات الحشرية لن تستطيع أن تتبادل الإشارات فيما بينها، وقد رأى العلماء أن نظاما يتضمن إفراز بعض المواد الكيميائية المتطايرة التى يمكن استخدامها لإيصال معلومة معينة أو لإصدار أمر ما. ولا شك أن مثل هذا النظام لن يتأثر بالظلام الدامس المنتشر في خلايا أو مستعمرات هذه الحشرات.

وتبين لهم أن هناك شفرة كيميائية خاصة بكل نوع من أنواع للإنذار الحشرات، وقد استن العلماء فهي تجاربهم خطة عمل غاية فهي البساطة، فقد قاموا أولا بدراسة أنواع الغدد التى توجد على جسم شغالات النمل، ثم قاموا بفصل للإنذار الغدد واحدة بعد الأخرى، واستخرجوا محتويات كل منها، ثم بدءوا فهي دراسة اثر محتويات كل غدة، على حدة، على مسلك أفراد الشغالات فهي مستعمرة النمل.

وقد أدى التعرف على هذه الغدد، ومعرفة الأثر المباشر الذى تحدثه محتويات كل منها إلى التوصل لفهم بعض الأسس التى يقوم عليها ذلك النظام الرائع الذى يسود مملكة النمل ويميزها من غيرها من الحشرات.

ومن أهم إفرازات هذه الغدد تلك المادة الكيميائية التى يتركها النمل على الأرض أثناء سيره والتى تستخدم دليلا للشغالات يساعدها على تحديد اتجاهها أثناء انتقالها من مكان لآخر ولذلك رأى تسمى عادة (مادة الأثر) أو ((مواد الأثر).

لقد تبين أن (مواد الأثر) تستخدم فهي زيادة نشاط الشغالات ودفعها إلى مزيد من العمل وهداية الشغالات إلى مواقع مناسبة لبناء عش جديد، ولكن فائدتها الرئيسية الغالبة هي إرشاد مجموعات الشغالات إلى مواقع الغذاء وهذه المواد تفرز بواسطة غدة خاصة تتصل بإبرة اللدغ الموجودة بمؤخرة النملة، وعندما تريد للإنذار النملة أن تضع مواد الأثر على الأرض رأى تفعل ذلك عادة بان تلمس الأرض بإبرتها الخلفية أثناء سيرها، وهى تفعل ذلك على فترات متقطعة بحيث تضع كل مرة قدرا ضئيلا من مادة الأثر، وينتج عن ذلك أن الإفراز الذى تضعه النملة على الأرض لا يكون على هيئة خط مستمر، بل يأخذ هيئة الخط المتقطع.

ويبدو أن الشغالات تنجذب انجذابا شديدا نحو مواد الأثر، فما أن تحس بوجود هذه المواد على الأرض، حتى تندفع بصورة تلقائية نحو هذا الخط المتقطع المرسوم بدقة، والذى يشبه ذلك الخط الأبيض المتقطع الذى تضعه إدارة المرور فهي منتصف الطريق.

ولعل ذلك يفسر لنا تلك الظاهرة التى نلحظها دائما، فجموع النمل تسير دائما بعضها وراء بعض، سواء على الأرض أو على الجدران. وهى تفعل ذلك فهي نظام شديد وكأنها تتبع فهي سيرها خطا وهميا. وفى الحقيقة لم يعد هذا الخط وهميا الأن ، بل اصبح أمرا واقعا، فان أفراد النمل تتبع ذلك الخط المتقطع من مادة الأثر على طول الطريق.

وقد لوحظ أن زيادة تركيز مواد الأثر يؤدى إلى حدوث ظاهرة غريبة لا تحدث إلا نادرا فهي مستعمرات النمل، يحدث فهي الحال ما شبه الهجرة الجماعية، فيتجه قسم كبير من هذه المستعمرة ومعه الملكة أحيانا فهي اتجاه هذه المحتويات تاركا القسم الأخر من المستعمرة وراءه. ويبدوا أن هذه الزيادة الهائلة فهي تركيز مواد الأثر يعتبر مؤشرا إلى زيادة إعداد سكان المستعمرة عما تستطيع أن تحتويه هذه المستعمرة فتحدث الهجرة الجماعية.

وكل ما يعرف عنها الآن أنها مادة طيارة إلى حد ما بمعنى أنها لا تبقى فهي مكانها طويلا، بل سريعا ما تتبخر ويضيع أثرها بمرور الوقت، كما اتضح انه يلزم وجود تركيز معين من هذه المادة حتى تتمكن الشغالات من الإحساس بها وتركيز مادة الأثر يبدأ فهي الاضمحلال فور وضعها على الأرض، ويضيع هذا الأثر نهائيا بعض مضى دقيقتين تقريبا على بدء وضعها على أي سطح من السطوح وهذه الخاصية الطيارة التى لمادة الأثر تعتبر من أهم مميزاتها، وهى تساعد على سهولة عمليات الاتصال داخل المستعمرة إلى حد كبير. فسرعة تطاير مادة الأثر وعدم بقائها على سطح الأرض لفترة طويلة يمنع تداخل الآثار القديمة التى سبق لأفراد النمل أن وضعتها فهي فترة سابقة ومع الآثار الجديدة، وذلك لان الآثار القديمة تكون قد تبخرت وانتهت منذ زمن طويل.

ولا شك أن هذه ميزة كبرى، فإنها تؤدى إلى عدم تداخل المعلومات القديمة مع المعلومات الجديدة وتمنع ما يمكن أن يحدث من لبس لأفراد الشغالات التى تخرج وراء الصيد الجديد.

وقد بينت التجارب التى أجريت فهي هذا المجال أن مادة الأثر تتوزع بطريقه متكافئة على طول خط الأثر، أي أن تركيزها يكون متساويا على طول المسار، رأى لا تكون مركزة فهي جزء منه ومخففة فهي جزء آخر، بل يكون توزيعها ثابتا على طول الطريق.

ولو أننا استطعنا أن نرى المادة الكيميائية التى تحدد الأثر لرأينا خطا متقطعا منتظما يتكون من عدة شرط متتابعة ومتساوية فهي الطول وفى السمك على طول المسار. ولا شك أن هذا التوزيع المتكافئ لمادة الأثر على طول

الجسم يخدم غرضا رئيسيا وهاما، فهو يساعد على اندفاع شغالات النمل نحو الهدف دون تردد وبسرعة ثابتة، وذلك لأن رائحة مادة الأثر تكون دائما ثابتة التركيز أمام للإنذار الشغالات.

وقد اتضح كذلك أن الأثر الفعال لمادة الأثر يتركز فوق خط المسار فقط، وان هذا التركيز يقل كثيرا بل يقل فجأة إذا خرجنا عن هذا الخط.

وتشبه مادة الأثر خط الحبر الرفيع المرسوم على الورق الجيد المصقول، ويعنى هذا أن مادة الأثر لا تنتشر فهي الفراغ المخيط بالمسار، بل تبقى مركزة فهي هذا الخط على الدوام حتى يتم تبخرها بعد ذلك بمرور الوقت.

ولا تتشابه مواد الأثر التى تستخدمها أنواع النمل المختلفة وبل يبدو أن كل نوع من أنواع النمل له موادها الكيميائية الخاصة به، فقد لوحظ أنت مواد الأثر المستخرجة من غدد جنس ما من النمل لا تؤثر فهي أفراد جنس آخر بل يبدو أن كل جنس من النمل له لغته الخاصة ومصطلحاته التى يتعامل بها. ولهذا لا يحدث خلط ما أو حتى اضطراب من أى نوع عندما يتقاطع خطا أثر لنوعين أو أكثر من أنواع النمل المختلفة، بل نجد أن كل فريق منها يستمر فهي تتبع مساره الخاص دون أن يلقى بالا أو حتى يشعر بمسار الفريق الأخر.

ويشبه هذا ما قد يحد ث عندما يتقابل فردان يتكلم كل منهما بلغته الخاصة ولا يفهم لغة الأخر. ولكن الفرق هنا كبير فان الإنسان يستطيع أن يتعلم أكثر من لغة بعضها الأخر؟

ولا يقف استخدام مواد الأثر على مملكة النمل فقط، بل هناك أنواع أخرى من الحشرات تقوم باستخدام بعض المواد المشابهة، ولكنها لم تدرس بعد بدرجة كافية كما درست مواد الأثر فهي حالة النمل.

مواد الإنذار

لا تعتبر مواد الأثر هى المواد الكيميائية الوحيدة التى يستخدمها النمل فهي عمليات الاتصال بين أفراده، بل هناك عدد آخر من هذه المواد تستخدمها الشغالات لتنظيم العمل داخل مملكة النمل الفائقة التنظيم.

وقد ساعد اكتشاف مواد الأثر على دفع بعض العلماء للبحث عن مثل هذه المواد الكيميائية التى تؤدى غرضا معينا وتقوم بدور فعال فهي حياة مستعمرة النمل، وانتهت هذه البحوث إلى اكتشاف بعض المواد الكيميائية الأخرى التى تستخدم فهي تبادل بعض الأخبار والمعلومات بين أفراد الشغالات مثل مواد الإنذار أو إنذاره بالوفاة.

وقد تصور بعض العلماء أن عملية الإنذار بالخطر تنتقل من نملة إلى أخرى عن طريق اللمس، بمعنى أن النملة إذا لمسنا ظهرها يصيبها الاضطراب فتندفع فهي طريقها لتصطدم بالنملة المجاورة لها التى تندفع هى الأخرى لتصطدم بغيرها، فيسرى الاضطراب فهي مجموعة النمل المحيطة بالنملة الأصلية كذلك اقترح علماء آخرون عملية انتقال الإنذار بالخطر تحدث عن طريق حركة قرون الاستشعار، فتقوم النملة التى تشعر بالخطر بالدق بقرون استشعارها بطريقة معينة على ظهور أفراد النمل المحيطة بها ثم تتكرر هذه الحالة من نملة إلى أخرى.

وهو ما يتنافى مع أن الإنذار بالخطر فى حقيقة الأمر لا يتعدى دائرة ضيقة، ويبقى محصورا دائما فى القطاع الصغير المحيط بالنملة التى شعرت بالخطر.

ولا يصلح لتفسير هذه الظاهرة إلا أن نفترض أن النملة المضطربة أو الخائفة عندما تشعر بالخطر تقوم بإفراز مادة كيميائية من نوع خاص تنتشر فهي القطاع المحيط بها وتعطى الإنذار بالخطر لجميع أفراد النمل الموجودة بهذا القطاع. وقد أثبتت التجارب صحة هذا الفرض، وأن النملة التى تشعر بالخطر أو يصيبها الانزعاج من مؤثر خارجي. تقوم بإفراز مادة كيميائية من الغدد الموجودة بجسمها، وهى غدد أخرى بخلاف الغدة التى تفرز مادة الأثر.

وقد تمكن العلماء من معرفة بعض مواد الإنذار التى يستخدمها النمل وتبين أنها مواد بسيطة التركيب وذات وزن جزيئي صغير، ومن أمثلتها السترال، والسترونيلال، والهبتانون، والدندرولاسين.

وهذه المواد الكيميائية لا أثر لها على الإنسان، ولا تثير فيه شيئا على الإطلاق وهى تعتبر بالنسبة له مواد ذات رائحة ذكية، بينما تحدث نفس هذه المركبات موجة من الهياج والذعر الهائل بين جموع النمل، بل هى تدفع مستعمرة من النمل إلى القيام بأعمال مذهلة فهي منتهى القسوة والعنف. ولعل هذا المثال يبين لنا الفرق الهائل بين إحساس الإنسان بما يحيط به، وبين شعور الحيوانات المختلفة بالعالم المحيط بها لان كلا منهما يعيش في عالمه الخاص.

وإذا درسنا الطريقة التى تنتشر بها للإنذار المواد لتبين لنا أن مادة الإنذار تتكون فهي الحقيقة من مادتين إحداها عبارة عن مادة سريعة التطاير وهى المادة الجاذبة وتوجد عادة بتركيز منخفض نسبيا تغلف المادة الأساسية وهى مادة الإنذار الحقيقية. وعلى ذلك فان محتويات هذه الغدد عندما تبدأ فهي التبخر تعطى مادتين متداخلين من البخار، بحيث تكون إحداهما داخل الأخرى،

وتكون كرة من المادة المتطايرة التى يبدو أن مهمة الاولى الأساسية اجتــذاب أفراد النمل إلى منطقة الاضطراب، بينما الثانية وهى مادة الإنــذار الحقيقــة تحدث الأثر المطلوب.

ويتلاشى أثر هذه المادة بعد فترة قــصيرة مــن اطلاقهــا وتعــد هــذه الخصائص العجيبة لمواد الإنذار إحدى المميزات الهامة التى تساعد أفراد النمل على تنظيم حياتها العادية.

ويمكننا القول بأن مواد الأثر أو مواد الإنذار لا تزيد عن كونها إحــدى مفردات لغة الكيمياء التى تتخاطب بها أفراد النمل وتتبــادل المعلومــات عــن طريقها، وهناك كثير من الشواهد التى تدل علــى أن جمــوع النمــل تــستخدم الإفرازات الكيميائية الأخرى في أغراض متشابهة، مثل الدعوة إلى تجمع سكان المستعمرة في مكان ما أو الدعوة إلى تناول الغــداء أو العنايــة بالملكــة، أو الاهتمام بصغار النمل غير الكاملة النمو إلى غير ذلك من الأعمال الأساسية التى يقوم بها النمل فهي حياته العادية.

والطريقة التى تعمل بها مثل هذه المواد التى تعطى الإنذار بــالخطر أو تدل على الوفاة أو تهدي إلى الأثر ومكان الغذاء هي أن شغالات النمل تــستقبل هذه المواد بقرون استشعارها في تجاويف خاصة، كما سنرى فيما بعــد عنــدما نتكلم عن نظرية الشم أو الإحساس بالروائح.

كذلك فقد بنيت المراقبة المستمرة لخلايا النحل أن هناك بعض الحــالات الخاصة التى تستعمل فيها لغة الكيمياء داخل الخلية، ومثال ذلك المادة الكيميائية التى تفرزها ملكة النحل لتنظيم دورة التكاثر في الخلية، وهى تفرز هذه المــادة من غددها الفكية. وعندما تتناول شغالات النحل هذه المادة تتوقف علــى الفــور عملية نمو المبايض في جسدها، كما تفقد كذلك قدرتها على بناء الخلايا الملكيــة التى تتربى فيها ملكات النحل، مما يساعد الملكة على إحكــام ســيطرتها علــى خليتها.

وكذلك تبين أن مملكة النحل قد تقوم باستخدام هذه المادة فــي أغــراض أخرى، رأى تستخدمها أحيانا أثناء طيران العرس لاجتذاب الذكور نحوها.

وعندما تنطلق هذه المادة من غدد الشغالات تنتشر في الهــواء المحــيط بالخلية فتقوم باجتذاب عدد كبير من الشغالات الأخرى نحو مصدر هذا الطعام، وبذلك تعتبر هذه المادة مكملة لرقصة النحل.

وهناك بعض المواد الأخرى التى تستخدم في دفع العــدوان ومقاومــة الدخلاء، فقد لوحظ أن عند تدخل أحد الغرباء في مملكة النحــل تقــوم بعــض

الشغالات القريبة بالتصدى لهذا الدخيل في الحال، وتبدأ في مهاجمتــه ولدغــه بعنف حتى يموت. ولا شك أن هذا إجراء دفاعي طبيعي، ولكن الشيء المدهش أن مئات الشغالات الأخرى تأتى مندفعة من كل حدب وصوب، وتقوم بمهاجمـــة هذا الدخيل بمنتهى العنف، وهى تستمر في لدغه بنفس العنف والقوة لمدة مـــا، حتى ولو كان العدو قد مات من أول لدغة.

لقد اتضح أن الشغالات الأولى التى تقوم بمهاجمة الدخيل انمـــا تفعـــل أمرين في وقت واحد، فهي تضع السم في جسد الدخيل عند لدغه كي تقتله، كما تضع في جسده كذلك قدرا ضئيلا جدا من افراز خاص له قـــدرة هائلـــة علـــى اجتذاب مئات من الشغالات.

جاذبات الجنس

تعتبر جاذبات الجنس من أهم المواد الكيميائية التـــى يـــستخدمها أفـــراد مملكة الحيوان. ومن المعروف أن مثل هذه المواد تفرزها بعض إناث الحشرات لاستكمال الذكور، وهذه المواد نوعية التأثير وتـــؤثر علـــى الجهـــاز العـــصبي المركزي فقط.

ويعود الفضل في هذا المجال إلى العالم الفرنسى (فابر) الذى عاش فـــي القرن التاسع عشر فقد قام هذا العالم بإجراء تجربة رائدة كانت هي رأس الحربة في تقدم العلم في هذا المضمار، وهي التي فتحت الأبواب أمام هذا الفرع مـــن العلم. فقد قام هذا العالم بوضع فراشة إحدى الحشرات التي تتغذى علـــى أوراق أشجار البلوط في قفص من القماش، ثم وضع هذا القفص بجـــوار النافـــذة فـــي منزله، وقد لاحظ (فابر) أنه بعد بضع ساعات تجمع حوالى ٦٠ ذكرا من ذكور هذا النوع من الحشرات حول القفص المحتوي على الأنثى وكأن هنـــاك نـــدا ء خفيا قد استطاع استحضار كل هذا العدد من الذكور.

وقد أثرت هذه الظاهرة الغريبة الدهشة إلى حد كبير وذلك لأن هذا النوع من الفراشات يعتبر نادرا ويصعب الحصول عليه وحتى رؤيته في الحقول فما الذي أدى إلى دعوة كل هذا العدد الكبير من ذكور هذه الحشرات التي انـــدفعت من كل حدب وصوب وكأن الأرض قد انشقت عنها، وما الذي جعلها تحوم حول هذا القفص المحتوي على الأنثى. ولم يستطع (فابر) أن يفسر هذه الظاهرة وظن في أول الأمر أن هذه الذكور قد استطاعت أن ترى الأنثى وهي داخل القفـــص. وأراد أن يتأكد من ذلك فوضع الأنثى في تجربة أخرى داخل قفص محكم مـــن الزجاج لعل ذلك يساعد على دعوة عدد أكبر من الذكور إذا استطاعت رؤيتهـــا

من وراء الزجاج الشفاف. وقد دهش هذا العالم لأن التجربة لم تنجح هذه المرة، فعلى الرغم من وضوح الأنثى داخل القفص الزجاجي إلا أنه لم يتجمع هذه المرة العدد المطلوب من الذكور بل لم يحم ذكر واحد حول هذا القفص وكأنه لا وجود للأنثى بداخله على الإطلاق. ولم يخطر ببال (فابر) أن رائحة الأنثى هي التي تجتذب الذكور، ففي الحالة الأولى كان القفص مصنوعا من القماش مما سمح بانتشار رائحة الأنثى في الجو المحيط بالقفص في حين أنه في الحالة الثانية تسبب القفص الزجاجي المحكم في منع رائحة الأنثى من الانتشار ولذلك لم تحس بها الذكور على الإطلاق.

ويبدو أن رائحة الأنثى كانت على درجة عالية من النفاذ حتى أنه عندما قام (فابر) بإخراج الأنثى من القفص استمر عدد كبير من الذكور في التجمع حول القفص الخالي رغم أنه كانت هناك بالحجرة التي أجريت بها التجربة بعض الروائح النفاذة الأخرى مثل دخان الطباق ورائحة النفتالين ورائحة كبريتيد الهيدروجين التي تشبه رائحة البيض الفاسد.

وقد بدأ استخدام المواد الجاذبة للجنس كسلاح ضد الحشرات عندما حاول العلماء مقاومة نوع معين من الفراش يطلق عليه اسم (فراش الجيبسي). فقد قام أحد الفرنسيين عام ١٨٦٩ بإحضار هذه الحشرة إلى الولايات المتحدة لكي يزاوج بينها وبين فراشة دودة القز لإنتاج نوع جديد من دود الحرير يستطيع أن يعيش ويتأقلم في أجواء الولايات المتحدة. ولم تكلل جهود هذا الرجل بالنجاح، بل الأدهى من ذلك أن بعض هذه الفراشات الجديدة قد استطاعت أن تفر منه واتجهت إلى الحقول المجاورة وبدأت في التكاثر بشكل هائل حتى أصبحت وباء يهدد جميع المزروعات والمحاصيل في ولاية نيوانجلاند، فقد انتشرت هذه الحشرة في جميع مزارع هذه الولاية الأمريكية وراحت تتغذى بكل شراهة على أوراق الأشجار وعلى المحاصيل دون تمييز، مما كلف هذه الولاية من الخسائر ما قدر بملايين الدولارات.

ونظرا للاجتياح الشديد لبعض هذه المواد الجاذبة للجنس لاستخدمها في مقاومة الآفات، وقلة ما يمكن فصله منها طبيعيا من إناث الحشرات بحيث لا تصلح للاستخدام على نطاق واسع، فقد فكر بعض العلماء المهتمين بهذا الموضوع في اختيار بعض المواد الكيميائية المعروفة والمختلفة معمليا واختبارها لعل بعضا منها يمكن استخدامه للقيام بنفس مهمة المواد الجاذبة للجنس، وحينئذ يمكن تحضير هذه المواد التي اجتازت الاختبار بكميات وافرة تصلح للاستخدام الحقلي.

وهناك كثير من المواد الكيميائية الأخرى – بخلاف المواد الجاذبة للجنس – التي استعملت في اجتذاب الحشرات ومثل بعض المواد الغذائية كمحاليل السكر والمواد النشوية وقطع الخيار وشرائح الموز وغيرها وبعض المواد الكيميائية البسيطة مثل كربونات النشادر التي تستطيع اجتذاب إناث حشرة الذباب المنزلي. وقد استخدمت في مقاومة بعض الآفات، وذلك بعد خلطها بمبيد حشري سريع التأثير، ووضعها في مصايد خاصة تتجمع فيها هذه الحشرات.

الباب الخامس

خاتمة:

يتبين مما سبق أن الخلية هي الوحدة الأساسية في بناء كل كائن حي. وتشبه كل خلية من هذه الخلايا معملا كيميائيا فريدا في نوعه، تتم فيه مئات من التفاعلات الكيميائية المعقدة التي تكرر نفسها على الدوام، وتتكون فيه مئات من أصناف الجزيئات الكيميائية التي يخدم كل منها غرضا معينا لا يحيد عنه.

ولا تتشابه كل الخلايا في جسد الكائن الحي، بل أن كثيرا منها له لغته الكيميائية الخاصة التي يتعامل بها، مما يؤدي في نهاية الأمر إلى تخصص هذه الخلايا وقيامها بوظيفة محددة في جسد الكائن الحي.

والخلية هي أصغر الوحدات في هذا الكون التي تعطينا مظاهر الحياة، ورغم ذلك فنحن لا نعرف شيئا عن الحياة نفسها. وعلى الرغم من أن كل خلية من خلايا جسد الكائن الحي تحمل في نواتها نفس العوامل الوراثية التي تحمل بين طياتها نفس الأوامر والتعليمات التي توجد بكل الخلايا الأخرى، إلا أننا نجد أن أجزاء كبيرة من هذه الرسائل تبقى معطلة في كل خلية. ولا تعمل كل خلية من هذه الخلايا إلا بقدر محدود من هذه الرسائل يتناسب مع الوظيفة التي تخصصت فيها. ويعنى ذلك أن كل خلايا جسد الكائن الحي لديها نفس كتاب الأوامر والتعليمات مكتوبا بنفس اللغة الكيميائية المتعارف عليها فيما بينها، إلا أن كل خلية فيها لا تقرأ إلا صفحة واحدة من هذا الكتاب وتعمل بما جاء فيها فقط، دون أن تلقي بالا إلى بقية صفحات هذا الكتاب.

ويمكن تشبيه جسد الكائن الحي في أرقى صوره – كما في الإنسان – بالمجتمع البشري الذي يتكون من ملايين من الأفراد، وبالرغم من تشابه أفراد هذا المجتمع في كثير من الصفات، إلا أن كلا منهم يؤدي وظيفة بعينها تتناسب مع ما أهل له ودرب عليه. ويتساءل كثير من العلماء عن الهدف الحقيقي من وجود مثل هذا النظام الفريد، المسمى بالحياة، والذي يختلف كل الاختلاف عن غيره من الموجودات في هذا الكون.

ونظرا لأن كل شئ في الخلية الحية يحدث طبقا لبرنامج مقرر من قبل، مسطور على جزيئات الحمض النووي DNA، فقد قوى الظن لدى كثير من العلماء، بأن هذا الحمض النووي قد يحمل أحد الجينات التي تخصصت في **إيقاف عملية الحياة ، وأن هذا (الجين) يحمل في ثناياه رسالة خاصة بهذا الأمر. ومن المعتقد أن هذا (الجين) يبقى ساكنا طوال حياة الكائن الحي، وتبقى الرسالة التي يحملها معطلة إلى حين، ولكنه في لحظة ما، ولسبب لانقضاء الأجل**

المكتوب والمقدر ، يبدأ في العمل، ويطلق الشرارة المناسبة فتبدأ عمليات الهـــدم والانحلال فعلها في جسد الكائن الحي حتى تفضي به إلى الموت. ولأن هذه حقيقة يعجز أمامها العقل فقد حلم علماء البيولوجيــا الجزيئيـــة *Molecular Biology* بأن يعطلوا عمل هذه الرسالة التي يحملها هذا (الجين)، لإطالة الحياة ولو إلى حين. وهو حلم من الأحلام الواهية.

كتبه أ.د/ حسين عبد المقصود على